Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	JAN 27	Source of Registration (SR) information in REGISTRY updated and searchable
NEWS	4	JAN 27	A new search aid, the Company Name Thesaurus, available in CA/CAplus
NEWS	5	FEB 05	German (DE) application and patent publication number format changes
NEWS	6	MAR 03	MEDLINE and LMEDLINE reloaded
NEWS	7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	8	MAR 03	FRANCEPAT now available on STN
NEWS	9	MAR 29	Pharmaceutical Substances (PS) now available on STN
NEWS	10	MAR 29	WPIFV now available on STN
NEWS	11	MAR 29	New monthly current-awareness alert (SDI) frequency in RAPRA
NEWS	12	APR 26	PROMT: New display field available
NEWS	13	APR 26	IFIPAT/IFIUDB/IFICDB: New super search and display field available
NEWS	14	APR 26	LITALERT now available on STN
NEWS	15	APR 27	NLDB: New search and display fields available
NEWS	16	May 10	PROUSDDR now available on STN
NEWS	17	May 19	PROUSDDR: One FREE connect hour, per account, in both May and June 2004
NEWS	18	May 12	EXTEND option available in structure searching
NEWS	19	May 12	Polymer links for the POLYLINK command completed in REGISTRY
NEWS	20	May 17	FRFULL now available on STN
NEWS	21	May 27	STN User Update to be held June 7 and June 8 at the SLA 2004 Conference
NEWS	22	May 27	New UPM (Update Code Maximum) field for more efficient patent SDIs in CAplus
NEWS	23	May 27	CAplus super roles and document types searchable in REGISTRY
NEWS	24	May 27	Explore APOLLIT with free connect time in June 2004

NEWS EXPRESS	MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

```
  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 17:42:55 ON 02 JUN 2004

=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'MEDLINE' ENTERED AT 17:43:19 ON 02 JUN 2004

FILE 'EMBASE' ENTERED AT 17:43:19 ON 02 JUN 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'BIOSIS' ENTERED AT 17:43:19 ON 02 JUN 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'SCISEARCH' ENTERED AT 17:43:19 ON 02 JUN 2004
COPYRIGHT 2004 THOMSON ISI

FILE 'CAPLUS' ENTERED AT 17:43:19 ON 02 JUN 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

=> s method
L1    12590928 METHOD

=> s l1 and inhibit?
L2      724225 L1 AND INHIBIT?

=> s l2 and aggregation or fibril formation
L3       20689 L2 AND AGGREGATION OR FIBRIL FORMATION

=> s l3 and motif
L4         263 L3 AND MOTIF

=> s l4 and identifying motif
L5           0 L4 AND IDENTIFYING MOTIF

=> s l4 and primary structure
L6           6 L4 AND PRIMARY STRUCTURE

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7           2 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> d l7 1-2 cbib abs

L7   ANSWER 1 OF 2  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:231741  Document No.: PREV200100231741. Complete amino acid sequence of
     kaouthiagin, a novel cobra venom metalloproteinase with two
     disintegrin-like sequences. Ito, Masayuki; Hamako, Jiharu; Sakurai,
     Yoshihiko; Matsumoto, Masanori; Fujimura, Yoshihiro; Suzuki, Masami;
     Hashimoto, Keiichiro; Titani, Koiti; Matsui, Taei [Reprint author].
     Division of Biomedical Polymer Science, Institute for Comprehensive
     Medical Science, Fujita Health University, Toyoake, Aichi, 470-1192,
     Japan. tmatsui@fujita-hu.ac.jp. Biochemistry, (April 10, 2001) Vol. 40,
     No. 14, pp. 4503-4511. print.
     CODEN: BICHAW. ISSN: 0006-2960. Language: English.
AB   The primary structure of kaouthiagin, a
     metalloproteinase from the venom of the cobra snake Naja kaouthia which
     specifically cleaves human von Willebrand factor (VWF), was determined by
     amino acid sequencing.  Kaouthiagin is composed of 401 amino acid residues
```

and one Asn-linked sugar chain. The sequence is highly similar to those of high-molecular mass snake venom metalloproteinases from viperid and crotalid venoms comprised of metalloproteinase, disintegrin-like, and Cys-rich domains. The metalloproteinase domain had a zinc-binding **motif** (HEXXHXXGXXH), which is highly conserved in the metzincin family. Kaouthiagin had an HDCD sequence in the disintegrin-like domain and uniquely had an RGD sequence in the Cys-rich domain. Metalloproteinase-inactivated kaouthiagin had no effect on VWF-induced platelet **aggregation** but still had an **inhibitory** effect on the collagen-induced platelet **aggregation** with an IC50 of 0.2 muM, suggesting the presence of disintegrin-like activity in kaouthiagin. To examine the effects of these HDCD and RGD sequences, we prepared synthetic peptides cyclized by an S-S linkage. Both the synthetic cyclized peptides (RAAKHDCDLPELC from the disintegrin-like domain and CFDLNMRGDDGSFC from the Cys-rich domain) had an **inhibitory** effect on collagen-induced platelet **aggregation** with IC50 values of apprx90 and apprx4.5 muM, respectively. The linear peptide (RAAKHDCDLPELC) and the cyclized peptide (CFDLNMRGEDGSFC) had little effect on collagen-induced platelet **aggregation.** These results suggest that kaouthiagin not only **inhibits** VWF-induced platelet **aggregation** by cleaving VWF but also disturbs the agonist-induced platelet **aggregation** by both the disintegrin-like domain and the RGD sequence in the Cys-rich domain. Furthermore, our results imply that the corresponding part of the Cys-rich domain in other snake venom metalloproteinases also has a synergistic disturbing effect on platelet **aggregation**, serving as a second disintegrin-like domain. This is the first report of an elapid venom metalloproteinase with two disintegrin-like sequences.

L7 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
2001051107. PubMed ID: 10940232. Review: immunoglobulin light chain amyloidosis--the archetype of structural and pathogenic variability. Bellotti V; Mangione P; Merlini G. (Department of Biochemistry, University of Pavia, Pavia, Italy.) Journal of structural biology, (2000 Jun) 130 (2-3) 280-9. Ref: 68. Journal code: 9011206. ISSN: 1047-8477. Pub. country: United States. Language: English.

AB AL amyloidosis is caused by deposition in target tissue of amyloid fibrils constituted by monoclonal immunoglobulin light chains. The amyloidogenic plasma cells derive from a transformed memory B cell that can be identified by anti-idiotype monoclonal antibodies. Comparison of the **primary structures** of amyloidogenic and nonamyloidogenic light chains does not show any common structural **motif** in the amyloidogenic variants but reveals peculiar replacements which can destabilize the folding state. Reduced folding stability now appears to be a unifying property of amyloidogenic light chains. The tendency of these proteins to populate a partially unfolded intermediate state is a key event in the self-association that progresses to the formation of oligomers and fibrils. The mechanism of organ damage caused by AL amyloid deposition is not known, but clinical findings suggest that the process of amyloid **fibril formation** itself exerts tissue toxic effects independently of the amount of amyloid deposited. Since the disease is caused by the neoplastic expansion of the plasma cell population synthesizing the amyloidogenic light chains, the clone represents the prime therapeutic target of conventional chemotherapy and experimental immunotherapy. In common with other types of amyloidosis the therapeutic strategy can take advantage of drugs able to improve the reabsorption of the amyloid deposits or able to bind and stabilize the light chain in the native-like folded state.

=> d his

(FILE 'HOME' ENTERED AT 17:42:55 ON 02 JUN 2004)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 17:43:19 ON 02 JUN 2004

L1 12590928 S METHOD
L2 724225 S L1 AND INHIBIT?
L3 20689 S L2 AND AGGREGATION OR FIBRIL FORMATION
L4 263 S L3 AND MOTIF
L5 0 S L4 AND IDENTIFYING MOTIF
L6 6 S L4 AND PRIMARY STRUCTURE
L7 2 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> s l4 and inhibitor
L8 80 L4 AND INHIBITOR

=> s l8 and protease inhibitor
L9 1 L8 AND PROTEASE INHIBITOR

=> d l9 cbib abs

L9 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:445879 Document No.: PREV200200445879. Savignygrin, a platelet **aggregation inhibitor** from the soft tick Ornithodoros savignyi, presents the RGD integrin recognition **motif** on the Kunitz-BPTI fold. Mans, Ben J.; Louw, Abraham I.; Neitz, Albert W. H. [Reprint author]. Department of Biochemistry, University of Pretoria, Pretoria, 0002, South Africa. albert.neitz@bioagric.up.ac.za. Journal of Biological Chemistry, (June 14, 2002) Vol. 277, No. 24, pp. 21371-21378. print.
CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

AB Savignygrin, a platelet **aggregation inhibitor** that possesses the RGD integrin recognition **motif**, has been purified from the soft tick Ornithodoros savignyi. Two isoforms with similar biological activities differ because of R52G and N60G in their amino acid sequences, indicating a recent gene duplication event. Platelet **aggregation** induced by ADP (IC50, 130 nM), collagen, the thrombin receptor-activating peptide, and epinephrine was **inhibited**, although platelets were activated and underwent a shape change. The binding of alpha-CD41 (P2) to platelets, the binding of purified alphaIIbbeta3 to fibrinogen, and the adhesion of platelets to fibrinogen was **inhibited**, indicating a targeting of the fibrinogen receptor. In contrast, the adhesion of osteosarcoma cells that express the integrin alphavbeta3 to vitronectin or fibrinogen was not **inhibited**, indicating the specificity of savignygrin toward alphaIIbbeta3. Savignygrin shows sequence identity to disagregin, a platelet **aggregation inhibitor** from the tick Ornithodoros moubata that lacks an RGD **motif.** The cysteine arrangement of savignygrin is similar to that of the bovine pancreatic trypsin **inhibitor** family of serine **protease inhibitors.** A homology model based on the structure of the tick anticoagulant peptide indicates that the RGD **motif** is presented on the substrate-binding loop of the canonical BPTI **inhibitors.** However, savignygrin did not **inhibit** the serine proteases fXa, plasmin, thrombin, or trypsin. This is the first report of a platelet **aggregation inhibitor** that presents the RGD **motif** using the Kunitz-BPTI protein fold.

=> s l3 and immunoglobulin light chain
L10 191 L3 AND IMMUNOGLOBULIN LIGHT CHAIN

=> s l10 and inhibitor
L11 19 L10 AND INHIBITOR

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12 15 DUP REMOVE L11 (4 DUPLICATES REMOVED)

=> d l12 1-15 cbib abs

L12 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2004:41732 Document No. 140:110124 Glycoproteins GPIa/IIa, GPIIb/IIIa and GPIb/IX to identify anti-platelet autoantibodies and **inhibitors** and for diagnosis and therapy of idiopathic thrombocytopenic purpura. Siegel, Donald L. (The Trustees of the University of Pennsylvania, USA). PCT Int. Appl. WO 2004005890 A2 20040115, 232 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US21304 20030703. PRIORITY: US 2002-PV394352 20020703; US 2002-PV411694 20020918.

AB The present invention relates to novel **methods** of identifying and producing an anti-platelet autoantibody. More preferably, the invention relates to identification and production of a human monoclonal anti-platelet autoantibody. Addnl., the invention relates to **methods** for producing and identifying **inhibitors** of an anti-platelet autoantibody binding with a platelet, or a platelet component e.g. glycoproteins GPIa/IIa, GPIIb/IIIa and GPIb/IX. Moreover, the invention relates to **methods** for treating or alleviating a disease, disorder or condition mediated by an anti-platelet autoantibody specifically binding with a platelet, or a component thereof, such as, but not limited to, idiopathic thrombocytopenic purpura, among others.

L12 ANSWER 2 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2004:356304 The Genuine Article (R) Number: 809KI. Pharmaceutical strategies against amyloidosis: Old and new drugs in targeting a "protein misfolding disease". De Lorenzi E; Giorgetti S; Grossi S; Merlini G; Caccialanza G; Bellotti V (Reprint). Univ Pavia, Dept Biochem, Biotechnol Lab, IRCCS Policlin San Matteo, Via Taramelli 3B, I-27100 Pavia, Italy (Reprint); Univ Pavia, Dept Biochem, Biotechnol Lab, IRCCS Policlin San Matteo, I-27100 Pavia, Italy; Univ Pavia, Dipartimento Chim Farmaceut, I-27100 Pavia, Italy; Ctr Studio & Cura Amiloidosi, Pavia, Italy. CURRENT MEDICINAL CHEMISTRY (APR 2004) Vol. 11, No. 8, pp. 1065-1084. Publisher: BENTHAM SCIENCE PUBL LTD. PO BOX 1673, 1200 BR HILVERSUM, NETHERLANDS. ISSN: 0929-8673. Pub. country: Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The group of diseases caused by abnormalities of the process of protein folding and unfolding is rapidly growing and includes diseases caused by loss of function as well as diseases caused by gain of function of misfolded proteins. Amyloidoses are caused by gain of function of certain proteins that lose their native structure and self-assemble into toxic insoluble, extracellular fibrils. This process requires the contribution of multiple factors of which only a few are established, namely the conformational modification of the amyloidogenic protein, protein's post-translational modifications and the co-deposition of glycosaminoglicans and of serum amyloid P component. In parallel with the exponential growth of biochemical data regarding the key events of the fibrillogenic process, several reports have shown that small molecules, through the interaction with either the amyloidogenic proteins or with the common constituents, can modify the kinetics of formation of amyloid fibrils or can facilitate amyloid reabsorption. These small molecules can be classified on the basis of their protein target and mechanism of action, according to the following properties. 1) molecules that stabilize the amyloidogenic protein precursor 2) molecules that prevent fibrillogenesis by acting on partially folded intermediates of the folding process as well as on low molecular weight oligomers populating the initial phase of **fibril formation** 3) molecules that

interact with mature amyloid fibrils and weaken their structural stability 4) molecules that displace fundamental co-factors of the amyloid deposits like glycosaminoglycans and serum amyloid P component and favor the dissolution of the fibrillar aggregate.

L12 ANSWER 3 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004101678 EMBASE Towards understanding the structure-function relationship of human amyloid disease. Dealwis C.; Wall J.. C. Dealwis, Dept. of Biochem. Cell./Molec. Biol., University of Tennessee, Knoxville, TN, United States. cdealwis@utk.edu. Current Drug Targets 5/2 (159-171) 2004.
Refs: 131.
ISSN: 1389-4501. CODEN: CDTUAU. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB **Immunoglobulin light chain** (LC) proteins exhibit the greatest sequence variability of all proteins associated with amyloid disease. The hallmark event in amyloidogenesis is a change in the secondary and/or tertiary structure or a normal, soluble protein, that fosters self-aggregation and **fibril formation.** The structural heterogeneity of light chain proteins has hampered understanding of the precise mechanisms involved in **fibril formation.** The development of effective therapeutics will be benefited by a fundamental understanding of mechanisms and structural prerequisites which govern amyloidogenesis. This review focuses on light chain (AL) amyloidosis resulting from the aggregation of κ and λ LCs. Specifically the thermodynamic and structural data of several WT and mutant amyloidogenic LCs have been carefully examined. Moreover, we discuss the importance of hydrophobic and ionic interactions on amyloidosis by comparing several available three-dimensional structures of amyloidogenic and highly homologous non-amyloidogenic proteins that can be destabilized to become amyloidogenic by site specific mutations.

L12 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2003:931387 Document No. 140:2559 **Method** for purifying denatured proteins having a desired disulfide bond configuration. Buus, Soren; Ferre, Henrik (Kobenhavns Universitet, Den.). PCT Int. Appl. WO 2003097669 A2 20031127, 41 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-DK324 20030515. PRIORITY: DK 2002-766 20020517.

AB The present invention relates to a **method** for production of a protein having a desired fold. This is especially achieved by subjecting a population of proteins to a separation step under non-reducing conditions. This allows for identification of a sub-population of proteins having the disulfide bond configuration resulting in a desired fold. Most often this will be the protein of proper structure and/or function. Thus, by using the novel **method** the purity of the protein having a desired fold can be increased as compared to the purity of a similar protein produced by a conventional **method.** Important aspect of the invention is a functional active MHC heavy chain protein obtainable by the above **method** and the use of a MHC heavy chain protein in anal. of peptide binding capacity. Oxidized species of murine and human recombinant MHC-I heavy chain monomers were separated by hydrophobic interaction chromatog. under nonreducing and denaturing conditions. One of these isomers was able to undergo efficient refolding and simultaneous peptide binding under acidic conditions.

L12 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:818235 Document No. 139:322283 **Methods** for production and use of mammalian complementarity determining region mimetibodies for diagnosis and therapy of human diseases. Heavner, George A.; Knight, David M.; Scallon, Bernard J.; Ghrayeb, John (Centocor, Inc., USA). PCT Int. Appl. WO 2003084477 A2 20031016, 97 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US9139 20030324. PRIORITY: US 2002-PV368791 20020329.

AB This invention pertains to **methods** for production and use of mammalian complementarity determining region (CDR) mimetibodies for diagnosis and therapy of human diseases. Genetic engineering, expression, and purification of human mimetibodies containing Ig fragments (CDR, variable, framework and/or constant region) as well as a ligand binding domain are disclosed in this invention. Peptides that mimic the activity of EPO, TPO, growth hormones, G-CSF, GM-CSF, IL-1ra, leptin, CTLA4, TRAIL, TGF-α and TGF-β are the focus of this genetic engineering. The aim of the invention is use of the purified recombinant proteins for diagnosis or treatment of anemia, immune or autoimmune disease, cancer, or infectious diseases. At the time of publication, claimed sequence nos. 997 to 1109 were missing, and claimed sequence nos. 984 to 996 were not clearly identified.

L12 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:6096 Document No. 138:49893 Protein **aggregation** assays and use in identification of therapeutic agents. Kondejewski, Les; Chakrabartty, Avijit; Qi, Xiao-Fei; Cashman, Neil (Caprion Pharmaceuticals Inc., Can.). PCT Int. Appl. WO 2003000853 A2 20030103, 107 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US19836 20020620. PRIORITY: US 2001-PV299849 20010620.

AB The invention features **methods** for identifying agents that modulate protein **aggregation** or stabilize protein conformation. The **methods** include an in vitro **aggregation** assay, a native state stabilization assay, a cell-based screening assay, and an animal-based screening assay. These **methods** can be used to identify agents useful for the treatment of conformational diseases resulting from **aggregation** of a protein.

L12 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:590596 Document No. 139:148471 Combinations of anti-tissue factor antibodies and anticoagulant and/or antiplatelet agents. Refino, Canio J.; Bunting, Stuart; Kirchhofer, Daniel (Genentech, Inc., USA). U.S. Pat. Appl. Publ. US 2003143225 A1 20030731, 64 pp., Cont.-in-part of U.S. Ser. No. 165,732. (English). CODEN: USXXCO. APPLICATION: US 2002-172785 20020613. PRIORITY: US 2001-802083 20010308; US 2002-165732 20020607.

AB The authors disclose anti-tissue factor (anti-TF) antibodies with enhanced anticoagulant potency and **methods** and means for identifying, producing and using such antibodies. The anti-TF antibodies of the present invention are designed to bind to an epitope comprising the C-terminal macromol. substrate binding region of TF. The invention also concerns **methods** of treating TF-VIIa related diseases or

disorders comprising administering anti-TF antibodies alone or in combination with at least one addnl. anticoagulant and/or anti-platelet agent.

L12 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2003:511840 Document No. 139:51623 Combination therapy using anti-tissue factor antibodies and anticoagulant and/or antiplatelet agents. Refino, Canio J.; Bunting, Stuart; Kirchhofer, Daniel (USA). U.S. Pat. Appl. Publ. US 2003124117 A1 20030703, 57 pp., Cont.-in-part of U.S. Ser. No. 802,083. (English). CODEN: USXXCO. APPLICATION: US 2002-165732 20020607. PRIORITY: US 2000-PV189775 20000316; US 2001-802083 20010308.
AB The invention concerns anti-tissue factor (anti-TF) antibodies with enhanced anticoagulant potency, and **methods** and means for identifying, producing and using such antibodies. The anti-TF antibodies of the present invention are designed to bind to an epitope comprising the C-terminal macromol. substrate binding region of TF. The invention also concerns **methods** of treating TF-VIIa related diseases or disorders comprising administering anti-TF antibodies alone or in combination with at least one addnl. anticoagulant and/or anti-platelet agent.

L12 ANSWER 9 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:572190 The Genuine Article (R) Number: 697RW. Structural transformations of oligomeric intermediates in the fibrillation of the **immunoglobulin light chain** LEN. Souillac P O; Uversky V N; Fink A L (Reprint). Univ Calif Santa Cruz, Dept Chem & Biochem, Santa Cruz, CA 95064 USA (Reprint). BIOCHEMISTRY (8 JUL 2003) Vol. 42, No. 26, pp. 8094-8104. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. ISSN: 0006-2960. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB LEN is a kappaIV **immunoglobulin light chain** variable domain from a patient suffering from multiple myeloma but with no evidence of amyloid fibrils. However, fibrils are formed when LEN solutions are agitated under mildly destabilizing conditions. Surprisingly, an inverse concentration dependence was observed on the kinetics of **fibril formation** because of the formation of off-pathway soluble oligomers at high protein concentration. Despite the fact that most of the protein is present in the off-pathway intermediates at relatively early times of aggregation, eventually all the protein forms fibrils. Thus, a structural rearrangement from the non fibril-prone off-pathway oligomers to a more fibril-prone species must occur. A variety of techniques were used to monitor changes in the size, secondary structure, solvent accessibility, and intrinsic stability of the oligomers, as a function of incubation time. The structural rearrangement was accompanied by a significant increase of disordered secondary structure, an increase in solvent accessibility, and a decrease in intrinsic stability of the soluble oligomeric species. We conclude that fibrils arise from the oligomers containing a less stable conformation of LEN, either directly or via dissociation. This is the first fibrillating system in which soluble off-pathway oligomeric intermediates have been shown to be the major transient species and in which fibrillation occurs from a relatively unfolded conformation present in these intermediates.

L12 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
2002:521933 Document No. 137:108286 Antibodies and fragments against epitopes present on cancer, metastatic or leukemia cells and platelets for diagnosis and therapy of tumor, metastasis, leukemia, autoimmune disease, and inflammation. Lazarovits, Janette; Hagai, Yocheved; Plaksin, Daniel; Vogel, Tikva; Nimrod, Abraham; Mar-Haim, Hagit; Szanthon, Ester; Richter, Tamar; Amit, Boaz; Kooperman, Lena; Peretz, Tuvia; Levanon, Avigdor (Bio-Technology General Corp., USA). PCT Int. Appl. WO 2002053700 A2 20020711, 310 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,

KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US49442 20011231. PRIORITY: US 2000-751181 20001229; US 2000-PV258948 20001229.

AB The present invention provides epitopes present on cancer cells and important in physiol. phenomena such as cell rolling, metastasis, and inflammation. Therapeutic and diagnostic **methods** and compns. using antibodies capable of binding to the epitopes are provided. The antibodies or fragments are capable of binding to, e.g. PSGL-1, fibrinogen γ prime, GP1bα, heparin, lumican, complement compound 4 (CC4), interalpha **inhibitor** and prothrombin. **Methods** and compns. according to the present invention can be used in diagnosis of and therapy for such diseases as cancer, including tumor growth and metastasis, leukemia, auto-immune disease, and inflammatory disease.

L12 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

2002:408803 Document No. 137:1503 Fusion protein of immunoglobulin heavy chain constant region and β-amyloid fragment as therapeutic agent for Alzheimer's disease. Gefter, Malcolm L.; Israel, David I.; Joyal, John L.; Gosselin, Michael (Praecis Pharmaceuticals Inc., USA). PCT Int. Appl. WO 2002042462 A2 20020530, 79 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US44581 20011127. PRIORITY: US 2000-PV253302 20001127; US 2000-PV250198 20001129; US 2000-PV257186 20001220.

AB The present invention provides therapeutic agents and **methods** of use thereof for treating an amyloidogenic disease, e.g., Alzheimer's disease. The therapeutic agents of the invention include compds. comprising the formula 1-L-P, wherein I is an Ig heavy chain constant region or fragment thereof (e.g., comprising the Fc region); L is a linker group or a direct bond; and P is a peptide capable of binding an amyloidogenic protein. It is believed that the P portion of the compds. of the invention will serve to bind an amyloidogenic protein, e.g., an amyloidogenic protein within an amyloid plaque, and the I portion of the compds. of the invention will serve to direct microglia to the amyloidogenic protein, which microglia may then internalize and degrade the amyloidogenic protein and the amyloid plaque. COS cells were transfected with DNA encoding various segments of β-amyloid flanked by the mouse IgGI Fc region. COS cells expressing the Fc Region of mouse IgG1 fused to amino acid residues 1-40, 1-42, 10-25, 16-30, 17-21, or 17-21-(A21L) of β-amyloid with or without an N-terminal triple glycine cap were resolved by SDS-PAGE in the absence of a reducing agent and examined by Western blot anal. The ability of a compound of the invention to modulate (e.g., **inhibit** or promote) the **aggregation** of natural β-AP when combined with the natural β-AP was examined using the Fibril binding assay. The results from this experiment (set forth in Figure 9), demonstrate that the compds. tested [e.g., PPI-1019, PPI-1621 and three different prepns. of Aβ(16-30)-Fc] are effective **inhibitors** of Aβ **aggregation.** The ability of Aβ(16-30)-Fc to clear amyloid plaques in a mouse model of Alzheimer's disease was assessed. The fusion protein was administered to a mouse transgenic for both the Swedish mutation of amyloid precursor protein and presenilin M146L by direct infusion into the cerebral cortex in one hemisphere. As indicated in Figure 10, the plaque burden at the site of infusion was significantly decreased compared to the controlled hemisphere.

L12 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 1

2002399251. PubMed ID: 12023282. Kinetics and energetics of assembly, nucleation, and growth of aggregates and fibrils for an amyloidogenic protein. Insights into transition states from pressure, temperature, and co-solute studies. Kim Yong-Sung; Randolph Theodore W; Stevens Fred J; Carpenter John F. (Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262, USA.) Journal of biological chemistry, (2002 Jul 26) 277 (30) 27240-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The transition states for prenucleation assembly, nucleation, and growth of aggregates and amyloid fibrils were investigated for a dimeric **immunoglobulin light chain** variable domain, employing pressure, temperature, and solutes as variables. Pressure-induced aggregation was nucleation-dependent and first-order in protein concentration and could be seeded. The insoluble aggregates were mixtures of amyloid fibrils and amorphous aggregates. Activation volumes, activation surface areas, and activation waters of hydration were larger for aggregate growth than for prenucleation assembly or nucleation, although activation free energies were similar for the three processes. Activation free energies for each of the transition states were dominated by the unfavorable free energy of solvation of newly exposed surfaces. Equilibrium dissociation and unfolding of the dimer showed a much larger volume change than those required to form the transition states for the three processes. Thus, the transition states for these steps are similar to the native state, and their formation requires only small structural perturbations. Finally, the presence of Congo red during amyloid **fibril formation** shortened lag times and caused pressure insensitivity of nucleation, suggesting that this compound or its analogs may not be effective as **inhibitors** of amyloidosis.

L12 ANSWER 13 OF 15 MEDLINE on STN

2001040337. PubMed ID: 11070162. Inhibition of amyloid fiber assembly by both BiP and its target peptide. Davis P D; Raffen R; Dul L J; Vogen M S; Williamson K E; Stevens J F; Argon Y. (Department of Pathology and Committee on Immunology, The University of Chicago, Illinois 60637, USA.) Immunity, (2000 Oct) 13 (4) 433-42. Journal code: 9432918. ISSN: 1074-7613. Pub. country: United States. Language: English.

AB **Immunoglobulin light chain** (LC) normally is a soluble, secreted protein, but some LC assemble into ordered fibrils whose deposition in tissues results in amyloidosis and organ failure. Here we reconstitute **fibril formation** in vitro and show that preformed fibrils can nucleate polymerization of soluble LC. This prion-like behavior has important physiological implications, since somatic mutations generate multiple related LC sequences. Furthermore, we demonstrate that **fibril formation** in vitro and aggregation of whole LC within cells are inhibited by BiP and by a synthetic peptide that is identical to a major LC binding site for BiP. We propose that LC form fibrils via an interprotein loop swap and that the underlying conformational change should be amenable to drug therapy.

L12 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1999:113797 Document No. 130:166800 Soluble fusion proteins of aggregate-forming proteins and the study of diseases associated with protein aggregate formation. Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian (Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany). PCT Int. Appl. WO 9906545 A2 19990211, 62 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-EP4811 19980731.

AB Fusion proteins of aggregate-forming proteins and solubilizing peptides are described for use in elucidating the mechanism, onset or progress of diseases associated with the formation of amyloid-like fibrils or protein aggregates. The **method** is for use in the study of neurol.

diseases such as Huntington's and Alzheimer's. The fusion proteins can also be used to screen for **inhibitors** of **aggregation** that may be of therapeutic use. Genes for a series of fusion proteins polyglutamine repeat expansion variants (20, 30, or 51 glutamine repeats) of huntingtin and glutathione-S-transferase were constructed by standard **methods** and manufactured in Escherichia coli using a hexahistidine for affinity purification The fusion proteins were soluble but cleavage of the 51 glutamine repeat variant (HD51) with trypsin led to the formation of insol. aggregates of the huntingtin. HD51 aggregated in vitro to form amyloid-like birefringent fibrils after liberation by trypsin cleavage, but the shorter repeat variants HD20 and HD30 did not do so. Similar effects were seen in vivo in COS-1 cells.

L12 ANSWER 15 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
94:592868 The Genuine Article (R) Number: PG110. POTENTIAL ROLE OF APOLIPOPROTEIN-E IN FIBRILLOGENESIS. GALLO G (Reprint); WISNIEWSKI T; CHOIMIURA N H; GHISO J; FRANGIONE B. NYU, TISCH HOSP, MED CTR, DEPT PATHOL, 550 1ST AVE, NEW YORK, NY, 10016 (Reprint). AMERICAN JOURNAL OF PATHOLOGY (SEP 1994) Vol. 145, No. 3, pp. 526-530. ISSN: 0002-9440. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Immunohistochemical and biochemical studies have demonstrated several different proteins in amyloid deposits that are not intrinsic components of the fibril itself but may play a role in their deposition and **fibril formation.** We compared the distribution of several amyloid-associated proteins, ie, amyloid P component, apolipoprotein-E, apolipoprotein-J, and vitronectin, in the deposits of several different amyloids, in particular light chain amyloid, with those in the deposits of nonamyloid monoclonal immunoglobulin, which may be considered a form of preamyloid disease. Although 100% of amyloid specimens (7 amyloid A, 15 **immunoglobulin light chain**, and 1 trans-thyretin) had amyloid P component and 100% had apolipoprotein-E (2 amyloid A, 10 **immunoglobulin light chain**, and 1 transthyretin) co-localized with the primary amyloid protein, none of the monoclonal nonamyloid cases (14 light chain deposition disease and 6 light and heavy chain deposition disease) had amyloid P component and only 1 of 11 had apolipoprotein-E. On the other hand, staining for apolipoprotein-J and vitronectin was positive in 100% of cases of amyloid and nonamyloid monoclonal deposits. The association between the presence of apolipoprotein-E and amyloid P component in the fibrillar from of monoclonal light chain deposits their absence in the nonfibrillar form of deposits suggest a role for these proteins in the process of fibrillogenesis. This lends support for the previously proposed concept that apolipoprotein-E functions as a pathological chaperone by altering the conformation of amyloidogenic proteins.

```
=> s l3 and transthyretin
L13          482 L3 AND TRANSTHYRETIN

=> s l13 and inhibitor
L14           99 L13 AND INHIBITOR

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15           47 DUP REMOVE L14 (52 DUPLICATES REMOVED)

=> s l15 and method steps
L16           12 L15 AND METHOD

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17           12 DUP REMOVE L16 (0 DUPLICATES REMOVED)

=> d l17 1-12 cbib abs
```

L17 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
2004:20807 Document No. 140:99589 Use of peptides derived from junctional adhesion molecules to permeabilize mucosa for improved efficiency of mucosal delivery of therapeutic compounds. Quay, Steven C. (Nastech Pharmaceutical Company, Inc., USA). PCT Int. Appl. WO 2004003145 A2 20040108, 426 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US19994 20030624. PRIORITY: US 2002-PV392512 20020628.

AB **Methods** of improving the permeability of mucosal epithelia to improve the efficiency of transmucosal delivery of drugs are described. Permeability is improved by modulating epithelial junction structure or physiol. of the mucosa using a peptide derived from one of the proteins involved in the junction, such as junctional adhesion mols. (JAMs), occludins, or claudins. The permeabilizing agent is typically a peptide or peptide analog or mimetic, often selected or derived from an extracellular domain of a mammalian JAM, occludin or claudin protein. Identification of candidate peptides derived from junctional adhesion mol. JAM-1, claudins and occludins is demonstrated. The effects of the peptides were tested in a com. airway epithelium model. Tests in adult male volunteers showed a significant improvement in the delivery of human interferon β across the nasal mucosa when a peptide derived from JAM-1 was included in an intranasal formulation.

L17 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
2003:6096 Document No. 138:49893 Protein **aggregation** assays and use in identification of therapeutic agents. Kondejewski, Les; Chakrabartty, Avijit; Qi, Xiao-Fei; Cashman, Neil (Caprion Pharmaceuticals Inc., Can.). PCT Int. Appl. WO 2003000853 A2 20030103, 107 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US19836 20020620. PRIORITY: US 2001-PV299849 20010620.

AB The invention features **methods** for identifying agents that modulate protein **aggregation** or stabilize protein conformation. The **methods** include an in vitro **aggregation** assay, a native state stabilization assay, a cell-based screening assay, and an animal-based screening assay. These **methods** can be used to identify agents useful for the treatment of conformational diseases resulting from **aggregation** of a protein.

L17 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:175023 Document No.: PREV200300175023. Energetic characteristics of the new **transthyretin** variant A25T may explain its atypical central nervous system pathology. Sekijima, Yoshiki; Hammarstrom, Per; Matsumura, Miyuki; Shimizu, Yuko; Iwata, Makoto; Tokuda, Takahiko; Ikeda, Shu-ichi; Kelly, Jeffery W. [Reprint Author]. Department of Chemistry, Skaggs Institute of Chemical Biology, Scripps Research Institute, 10550 N. Torrey Pines Road, BCC 265, La Jolla, CA, 92037, USA. jwk@scripps.edu. Laboratory Investigation, (March 2003) Vol. 83, No. 3, pp. 409-417. print. CODEN: LAINAW. ISSN: 0023-6837. Language: English.

AB **Transthyretin** (TTR) is a tetrameric protein that must misfold to

form amyloid fibrils. Misfolding includes rate-limiting tetramer dissociation, followed by fast tertiary structural changes that enable **aggregation.** Amyloidogenesis of wild-type (WT) TTR causes a late-onset cardiac disease called senile systemic amyloidosis. The **aggregation** of one of >80 TTR variants leads to familial amyloidosis encompassing a collection of disorders characterized by peripheral neuropathy and/or cardiomyopathy. Prominent central nervous system (CNS) impairment is rare in TTR amyloidosis. Herein, we identify a new A25T TTR variant in a Japanese patient who presented with CNS amyloidosis at age 42 and peripheral neuropathy at age 44. The A25T variant is the most destabilized and fastest dissociating TTR tetramer published to date, yet, surprising, disease onset is in the fifth decade. Quantification of A25T TTR in the serum of this heterozygote reveals low levels relative to WT, suggesting that protein concentration influences disease phenotype. Another recently characterized TTR CNS variant (D18G TTR) exhibits strictly analogous characteristics, suggesting that instability coupled with low serum concentrations is the signature of CNS pathology and protects against early-onset systemic amyloidosis. The low A25T serum concentration may be explained either by impaired secretion from the liver or by increased clearance, both scenarios consistent with A25T's low kinetic and thermodynamic stability. Liver transplantation is the only known treatment for familial amyloid polyneuropathy. This is a form of gene therapy that removes the variant protein from serum preventing systemic amyloidosis. Unfortunately, the choroid plexus would have to be resected to remove A25T from the CSF-the source of the CNS TTR amyloid. Herein we demonstrate that small-molecule tetramer stabilizers represent an attractive therapeutic strategy to **inhibit** A25T misfolding and CNS amyloidosis. Specifically, 2-((3,5-dichlorophenyl)amino)benzoic acid is an excellent **inhibitor** of A25T TTR amyloidosis in vitro.

L17 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2002:637943 Document No. 137:179845 Drug screening **method** for the treatment of human disease associated with protein misfolding using yeast system. Lindquist, Susan; Krobitsch, Sylvia; Outeiro, Tiago (University of Chicago, USA). PCT Int. Appl. WO 2002065136 A2 20020822, 93 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US4632 20020215. PRIORITY: US 2001-PV269157 20010215.

AB Screening **methods** for identifying substances that provide therapeutic value for various diseases associated with protein misfolding are provided. Genetic and chemical screening **methods** are provided using a yeast system. The **methods** of the invention provide a rapid and cost-effective **method** to screen for compds. that prevent protein misfolding and/or protein **fibril formation** and/or protein **aggregation** which includes numerous neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, Huntington's disease as well as non-neuronal diseases such as type 2 diabetes.

L17 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2002:408803 Document No. 137:1503 Fusion protein of immunoglobulin heavy chain constant region and β-amyloid fragment as therapeutic agent for Alzheimer's disease. Gefter, Malcolm L.; Israel, David I.; Joyal, John L.; Gosselin, Michael (Praecis Pharmaceuticals Inc., USA). PCT Int. Appl. WO 2002042462 A2 20020530, 79 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,

KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US44581 20011127. PRIORITY: US 2000-PV253302 20001127; US 2000-PV250198 20001129; US 2000-PV257186 20001220.

AB The present invention provides therapeutic agents and **methods** of use thereof for treating an amyloidogenic disease, e.g., Alzheimer's disease. The therapeutic agents of the invention include compds. comprising the formula 1-L-P, wherein I is an Ig heavy chain constant region or fragment thereof (e.g., comprising the Fc region); L is a linker group or a direct bond; and P is a peptide capable of binding an amyloidogenic protein. It is believed that the P portion of the compds. of the invention will serve to bind an amyloidogenic protein, e.g., an amyloidogenic protein within an amyloid plaque, and the I portion of the compds. of the invention will serve to direct microglia to the amyloidogenic protein, which microglia may then internalize and degrade the amyloidogenic protein and the amyloid plaque. COS cells were transfected with DNA encoding various segments of β-amyloid flanked by the mouse IgGI Fc region. COS cells expressing the Fc Region of mouse IgG1 fused to amino acid residues 1-40, 1-42, 10-25, 16-30, 17-21, or 17-21-(A21L) of β-amyloid with or without an N-terminal triple glycine cap were resolved by SDS-PAGE in the absence of a reducing agent and examined by Western blot anal. The ability of a compound of the invention to modulate (e.g., **inhibit** or promote) the **aggregation** of natural β-AP when combined with the natural β-AP was examined using the Fibril binding assay. The results from this experiment (set forth in Figure 9), demonstrate that the compds. tested [e.g., PPI-1019, PPI-1621 and three different prepns. of Aβ(16-30)-Fc] are effective **inhibitors** of Aβ **aggregation.** The ability of Aβ(16-30)-Fc to clear amyloid plaques in a mouse model of Alzheimer's disease was assessed. The fusion protein was administered to a mouse transgenic for both the Swedish mutation of amyloid precursor protein and presenilin M146L by direct infusion into the cerebral cortex in one hemisphere. As indicated in Figure 10, the plaque burden at the site of infusion was significantly decreased compared to the controlled hemisphere.

L17 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2002:240731 Document No. 136:257287 Compounds and **methods** for diagnosing and treating amyloid-related conditions. Raub, Thomas J.; Tanis, Steven P.; Buhl, Allen Edwin; Carter, Donald Bainbridge; Bandiera, Tiziano; Lansen, Jacqueline; Pellerano, Cesare; Savini, Luisa (Pharmacia & Upjohn Company, USA; Pharmacia & Upjohn S.p.A.). PCT Int. Appl. WO 2002024652 A1 20020328, 56 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US29010 20010917. PRIORITY: US 2000-PV234611 20000922; US 2000-667357 20000922.

AB The invention provides **methods** for diagnosing and treating amyloid-related conditions and compds. useful for the same. The invention provides for detecting, imaging, monitoring, diagnosing, and treating conditions characterized by the binding or **aggregation** of amyloid fibrils. More particularly, the invention relates to using quinolinehydrazone compds. for diagnosing and treating amyloidotic conditions and also as an antioxidant. Examples are provided showing that 4-methyl-7-methoxy-2-(4-quinolylmethylenehydrazino)quinoline is suitable for fluorescence detection of amyloid plaque and has antioxidant activity.

L17 ANSWER 7 OF 12 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:6042 The Genuine Article (R) Number: 624LJ. Synthesis and structural analysis of the N-terminal domain of the thyroid hormone-binding protein **transthyretin.** Wilce J A; Daly N L; Craik D J (Reprint). Univ Queensland, Inst Mol Biosci, Brisbane, Qld 4072, Australia (Reprint); Univ Western Australia, Dept Biochem Chem, Nedlands, WA 6009, Australia; Univ Queensland, ARC Special Res Ctr Funct & Appl Genom, Brisbane, Qld 4072, Australia. CLINICAL CHEMISTRY AND LABORATORY MEDICINE (DEC 2002) Vol. 40, No. 12, pp. 1221-1228. Publisher: WALTER DE GRUYTER & CO. GENTHINER STRASSE 13, D-10785 BERLIN, GERMANY. ISSN: 1434-6621. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Transthyretin** (TTR) is a 55 kDa protein responsible for the transport of thyroid hormones and retinol in human serum. Misfolded forms of the protein are implicated in the amyloid diseases familial amyloidotic polyneuropathy and senile systemic amyloidosis. Its folding properties and stabilization by ligands are of current interest due to their importance in understanding and combating these diseases. To assist in such studies we developed a **method** for the solid phase synthesis of the monomeric unit of a TTR analogue and its folding to form a functional 55 kDa tetramer. The monomeric unit of the protein was chemically synthesized in three parts, comprising amino acid residues 151, 5499 and 102127, and ligated using chemoselective thioether ligation chemistry. The synthetic protein was folded and assembled to a tetrameric structure in the presence of the TTRs native ligand, thyroxine, as shown by gel filtration chromatography, native gel electrophoresis, TTR antibody recognition and thyroid hormone binding. In the current study the solution structure of the first of these fragment peptides, TTR(151) is examined to determine its intrinsic propensity to form beta-sheet structure, potentially involved in amyloid **fibril formation** by TTR. Despite the presence of extensive beta-structure in the native form of the protein, the Nterminal fragment adopts an essentially random coil conformation in solution.

L17 ANSWER 8 OF 12 MEDLINE on STN
2001250841. PubMed ID: 11344299. Evaluating the binding selectivity of **transthyretin** amyloid fibril **inhibitors** in blood plasma.
Purkey H E; Dorrell M I; Kelly J W. (Department of Chemistry and The Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, MB12, La Jolla, CA 92037, USA.)
Proceedings of the National Academy of Sciences of the United States of America, (2001 May 8) 98 (10) 5566-71. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB **Transthyretin** (TTR) tetramer dissociation and misfolding facilitate assembly into amyloid fibrils that putatively cause senile systemic amyloidosis and familial amyloid polyneuropathy. We have previously discovered more than 50 small molecules that bind to and stabilize tetrameric TTR, inhibiting amyloid **fibril formation** in vitro. A **method** is presented here to evaluate the binding selectivity of these **inhibitors** to TTR in human plasma, a complex biological fluid composed of more than 60 proteins and numerous small molecules. Our immunoprecipitation approach isolates TTR and bound small molecules from a biological fluid such as plasma, and quantifies the amount of small molecules bound to the protein by HPLC analysis. This approach demonstrates that only a small subset of the **inhibitors** that saturate the TTR binding sites in vitro do so in plasma. These selective **inhibitors** can now be tested in animal models of TTR amyloid disease to probe the validity of the amyloid hypothesis. This **method** could be easily extended to evaluate small molecule binding selectivity to any protein in a given biological fluid without the necessity of determining or guessing which other protein components may be competitors. This is a central issue to understanding the distribution, metabolism, activity, and toxicity of potential drugs.

L17 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:333843 Document No.: PREV200100333843. Understanding amyloidogenicity in vitro and in vivo towards therapeutic intervention. Kelly, Jeffery W. [Reprint author]. Department of Chemistry, Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA, 92037, USA. jkelly@scripps.edu. Abstracts of Papers American Chemical Society, (2001) Vol. 221, No. 1-2, pp. ORGN 253. print.
Meeting Info.: 221st National Meeting of the American Chemical Society. San Diego, California, USA. April 01-05, 2001. American Chemical Society.
CODEN: ACSRAL. ISSN: 0065-7727. Language: English.

L17 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
1999:113797 Document No. 130:166800 Soluble fusion proteins of aggregate-forming proteins and the study of diseases associated with protein aggregate formation. Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian (Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany). PCT Int. Appl. WO 9906545 A2 19990211, 62 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-EP4811 19980731.
AB Fusion proteins of aggregate-forming proteins and solubilizing peptides are described for use in elucidating the mechanism, onset or progress of diseases associated with the formation of amyloid-like fibrils or protein aggregates. The **method** is for use in the study of neurol. diseases such as Huntington's and Alzheimer's. The fusion proteins can also be used to screen for **inhibitors** of **aggregation** that may be of therapeutic use. Genes for a series of fusion proteins polyglutamine repeat expansion variants (20, 30, or 51 glutamine repeats) of huntingtin and glutathione-S-transferase were constructed by standard **methods** and manufactured in Escherichia coli using a hexahistidine for affinity purification The fusion proteins were soluble but cleavage of the 51 glutamine repeat variant (HD51) with trypsin led to the formation of insol. aggregates of the huntingtin. HD51 aggregated in vitro to form amyloid-like birefringent fibrils after liberation by trypsin cleavage, but the shorter repeat variants HD20 and HD30 did not do so. Similar effects were seen in vivo in COS-1 cells.

L17 ANSWER 11 OF 12 MEDLINE on STN
1999007248. PubMed ID: 9789022. Inhibiting **transthyretin** conformational changes that lead to amyloid **fibril formation.** Peterson S A; Klabunde T; Lashuel H A; Purkey H; Sacchettini J C; Kelly J W. (Department of Chemistry and Skaggs Institute of Chemical Biology, Scripps Research Institute, 10550 North Torrey Pines Road MB 12, La Jolla, CA 92037, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1998 Oct 27) 95 (22) 12956-60. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Insoluble protein fibrils resulting from the self-assembly of a conformational intermediate are implicated as the causative agent in several severe human amyloid diseases, including Alzheimer's disease, familial amyloid polyneuropathy, and senile systemic amyloidosis. The latter two diseases are associated with **transthyretin** (TTR) amyloid fibrils, which appear to form in the acidic partial denaturing environment of the lysosome. Here we demonstrate that flufenamic acid (Flu) inhibits the conformational changes of TTR associated with amyloid **fibril formation.** The crystal structure of TTR complexed with Flu demonstrates that Flu mediates intersubunit hydrophobic interactions and intersubunit hydrogen bonds that stabilize the normal tetrameric fold of TTR. A small-molecule **inhibitor** that stabilizes the normal conformation of a protein is desirable as a possible approach to treat amyloid diseases. Molecules such as Flu also provide the means to rigorously test the amyloid hypothesis, i.e., the apparent causative role of amyloid fibrils in amyloid disease.

L17 ANSWER 12 OF 12 MEDLINE on STN

97047186. PubMed ID: 8892106. Relative efficacies of amyloid beta peptide (A beta) binding proteins in A beta **aggregation.** Webster S; Rogers J. (L.J. Roberts Center for Alzheimer's Research, Sun Health Research Institute, Arizona 85372, USA.) Journal of neuroscience research, (1996 Oct 1) 46 (1) 58-66. Journal code: 7600111. ISSN: 0360-4012. Pub. country: United States. Language: English.

AB The **aggregation** of amyloid beta peptide (A beta) into its fibrillar, cross beta-pleated configuration is generally viewed as a critical event in the pathophysiology of Alzheimer's disease (AD). A diverse group of molecules, the A beta binding proteins, has been evaluated for their effects on this process. However, most of these studies have used micromolar or greater reagent concentrations, and their different **methods** have not permitted quantitative comparisons of the efficacy of different A beta binding proteins in augmenting or **inhibiting aggregation.** In the present work we have undertaken a coherent analysis using fluorimetry of thioflavin T-stained experimental solutions. The complement protein C1q, serum amyloid P, and **transthyretin** significantly enhanced the formation of precipitable, cross beta-pleated aggregates in solutions of 800 nM A beta 1-42. Under these same experimental conditions, alpha 1-antichymotrypsin had no significant effect on the **aggregation** process, and both the E3 and E4 isoforms of apolipoprotein E were significant **inhibitors.** There was a non-significant trend toward the E3 isoform exhibiting greater **inhibition** than the E4 isoform. Of the **aggregation**-facilitating molecules, C1q was substantially and significantly the most potent.

```
=> s l3 and beta-2 microglobulin
L18          183 L3 AND BETA-2 MICROGLOBULIN

=> s l18 and serine protease inhibitor
L19            0 L18 AND SERINE PROTEASE INHIBITOR

=> s l18 and inhibitor
L20            4 L18 AND INHIBITOR

=> dup remove l20
PROCESSING COMPLETED FOR L20
L21            4 DUP REMOVE L20 (0 DUPLICATES REMOVED)

=> d l21 1-4 cbib abs

L21  ANSWER 1 OF 4  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2004149212  EMBASE  Pharmaceutical strategies against amyloidosis: Old and new
     drugs in targeting a "protein misfolding disease". De Lorenzi E.;
     Giorgetti S.; Grossi S.; Merlini G.; Caccialanza G.; Bellotti V.. V.
     Bellotti, Department of Biochemistry, University of Pavia, Via Taramelli
     3/b, 27100 Pavia, Italy. vbellot@unipv.it. Current Medicinal Chemistry
     11/8 (1065-1084)  2004.
     Refs: 224.
     ISSN: 0929-8673. CODEN: CMCHE7. Pub. Country: Netherlands. Language:
     English. Summary Language: English.
AB   The group of diseases caused by abnormalities of the process of protein
     folding and unfolding is rapidly growing and includes diseases caused by
     loss of function as well as diseases caused by gain of function of
     misfolded proteins. Amyloidoses are caused by gain of function of certain
     proteins that lose their native structure and self-assemble into toxic
     insoluble, extracellular fibrils. This process requires the contribution
     of multiple factors of which only a few are established, namely the
     conformational modification of the amyloidogenic protein, protein's
     post-translational modifications and the co-deposition of
     glycosaminoglicans and of serum amyloid P component. In parallel with the
     exponential growth of biochemical data regarding the key events of the
```

fibrillogenic process, several reports have shown that small molecules, through the interaction with either the amyloidogenic proteins or with the common constituents, can modify the kinetics of formation of amyloid fibrils or can facilitate amyloid reabsorption. These small molecules can be classified on the basis of their protein target and mechanism of action, according to the following properties. 1) molecules that stabilize the amyloidogenic protein precursor 2) molecules that prevent fibrillogenesis by acting on partially folded intermediates of the folding process as well as on low molecular weight oligomers populating the initial phase of **fibril formation** 3) molecules that interact with mature amyloid fibrils and weaken their structural stability 4) molecules that displace fundamental co-factors of the amyloid deposits like glycosaminoglycans and serum amyloid P component and favor the dissolution of the fibrillar aggregate. .COPYRGT. 2004 Bentham Science Publishers Ltd.

L21 ANSWER 2 OF 4 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:283849 The Genuine Article (R) Number: 416DL. Detection of two partially structured species in the folding process of the amyloidogenic protein **beta 2-microglobulin.** Chiti F; Mangione P; Andreola A; Giorgetti S; Stefani M; Dobson C M; Bellottl V; Taddei N (Reprint). Univ Florence, Dipartimento Sci Biochim, Viale Morgagni 50, I-50134 Florence, Italy (Reprint); Univ Florence, Dipartimento Sci Biochim, I-50134 Florence, Italy; Univ Pavia, Dipartimento Biochim, I-27100 Pavia, Italy; IRCCS, Policlin San Matteo, Biotechnol Labs, I-27100 Pavia, Italy; Univ Oxford, New Chem Lab, Oxford Ctr Mol Sci, Oxford OX1 3QT, England. JOURNAL OF MOLECULAR BIOLOGY (16 MAR 2001) Vol. 307, No. 1, pp. 379-391. Publisher: ACADEMIC PRESS LTD. 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND. ISSN: 0022-2836. Pub. country: Italy; England. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB beta2-Microglobulin is a small, major histocompatibility complex class I-associated protein that undergoes aggregation and accumulates as amyloid deposits in human tissues as a consequence of long-term haemodialysis. The folding process of this amyloidogenic protein has been studied in vitro by diluting the guanidine hydrochloride-denatured protein in refolding buffer at pH 7.4 and monitoring the folding process by means of a number of spectroscopic probes that allow the native structure of the protein to be detected as it develops. These techniques include fluorescence spectroscopy, far and near-UV circular dichroism, 8-anilino-1-naphthalenesulfonic acid binding and double jump assays. All spectroscopic probes indicate that a significant amount of structure forms within the dead-time of stopped-flow measurements (<5 ms). The folding reaction goes to completion through a fast phase followed by a slow phase, whose rate constants are ca 5.1 and 0.0030 s(-1) in water, respectively. Unfolding-folding double jump experiments, together with the use of peptidyl prolyl isomerase, reveal that the slow phase of folding of < **beta>2-microglobulin** is not fundamentally determined by cis/trans isomerisation of X-Pro peptide bonds. Other folding-unfolding double jump experiments also suggest that the fast and slow phases of folding are not related to independent folding of different populations of protein molecules. Rather, we provide evidence for a sequential mechanism of folding where denatured beta2-microglobulin collapses to an ensemble of partially folded conformations (I-1) which fold subsequently to a more highly structured species (I-2) and, finally, attain the native state. The partially folded species I-2 appears to be closely similar to previously studied amyloidogenic forms of beta2-microglobulin, such as those adopted by the protein at mildly acid pH values and by a variant with six residues deleted at the N terminus. Since amyloid formation in vivo originates from partial denaturation of beta2-microglobulin under conditions favouring the folding process, the long-lived, partially structured species detected here might be significantly populated under some physiological conditions and hence might play an important role in the process of amyloid formation. (C) 2001 Academic Press.

L21 ANSWER 3 OF 4 MEDLINE on STN
2001428412. PubMed ID: 11475719. Comparative study on effect of Panax notoginseng and ticlid in treating early diabetic nephropathy. Lang J; Cao H; Wei A. (Foshan TCM Hospital, Guangdong 528000.) Zhongguo zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi = Chinese journal of integrated traditional and Western medicine / Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu ban, (1998 Dec) 18 (12) 727-9. Journal code: 9211576. ISSN: 1003-5370. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To explore the ameliorative effect and mechanism of Panax notoginseng (PNG) and ticlid in treating early diabetic nephropathy (DN). **METHODS**: Fifty-eight patients were divided randomly into two groups, 28 patients of the ticlid group treated with ticlid 250 mg orally, once a day and 30 patients of the PNG group treated with PNG 8 ml in 250 ml of normal saline intravenous drip once a day. The therapeutic effect and relative indexes of the two groups were observed and compared. RESULTS: After treatment, in both groups, the thromboxane B2 markedly reduced and was more prominent in the ticlid group ($P < 0.05$), while the 6-keto-prostaglandin F1 alpha increased obviously, so as to cause a significant lowering of T/K ratio, $P < 0.01$. Levels of urinary albumin, **beta 2 microglobulin** and blood alpha 1 microglobulin of both groups were lowered significantly, $P < 0.01$. A significant positive linear correlation was found in the ticlid group between urinary albumin and T/K ratio ($r = 0.41$, $P < 0.01$), as well as in blood alpha 1 microglobulin with T/K ratio ($r = 0.34$, $P < 0.05$), while it was not found in the PNG group. CONCLUSION: Ticlid and PNG were beneficial to resume the balance of T/K and improve microcirculation, reduce whole blood viscosity and decrease urinary albumin so as to retard the progress of DN.

L21 ANSWER 4 OF 4 MEDLINE on STN
87302703. PubMed ID: 2887330. Lymphocyte transglutaminase function may be impaired in type 2 diabetes mellitus. Berntorp E; Berntorp K; Seiving B; Stenberg P. Diabetes research (Edinburgh, Lothian), (1987 May) 5 (1) 29-33. Journal code: 8502339. ISSN: 0265-5985. Pub. country: SCOTLAND: United Kingdom. Language: English.

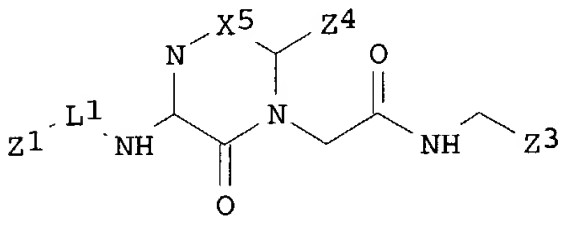
AB A **method** for capping of **beta 2-microglobulin** involving the transglutaminase **inhibitor** monodansylthiacadaverine was applied to lymphocytes from 17 patients with Type 2 diabetes mellitus and from a matched control group of 16 normoglycaemic healthy subjects. Monodansylthiacadaverine strongly **inhibited** the capping, which points to the involvement of transglutaminase in the redistribution of **beta 2-microglobulin** on the cell surface. The **inhibition** was more pronounced in lymphocytes from diabetic patients, indicating impaired transglutaminase function in Type 2 diabetes mellitus.

```
=> s l3 adn protease inhibitor
MISSING OPERATOR L3 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l3 and protease inhibitor
L22         201 L3 AND PROTEASE INHIBITOR

=> s l22 and serine protease inhibitor
L23          39 L22 AND SERINE PROTEASE INHIBITOR

=> dup remove l23
PROCESSING COMPLETED FOR L23
L24          27 DUP REMOVE L23 (12 DUPLICATES REMOVED)

=> d l24 1-27 cbib abs
```

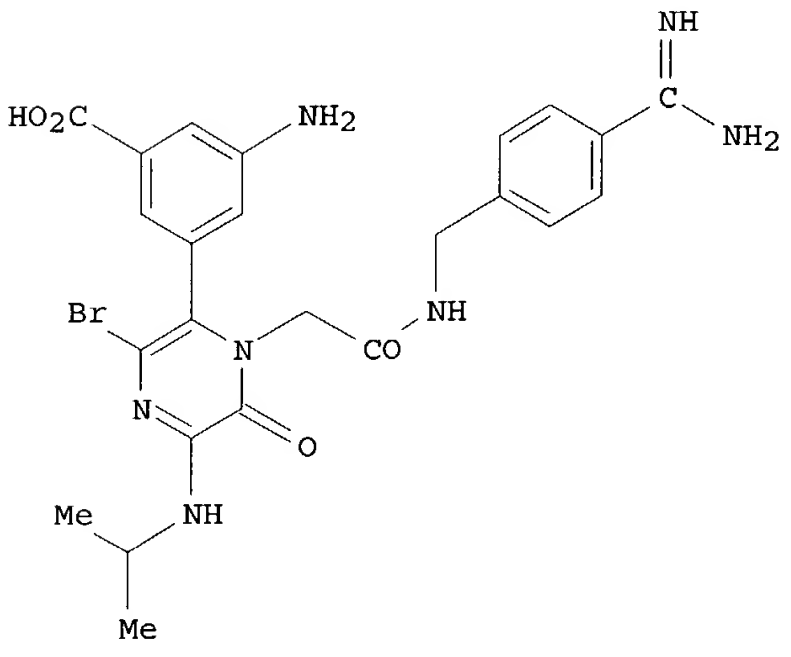
L24 ANSWER 1 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN

2003:282545 Document No. 138:304305 Preparation of 6-membered unsaturated heterocyclic compounds such as amidinobenzyl oxopyrazinylacetamides useful for selective **inhibition** of serine proteases of the coagulation cascade. South, Michael S.; Webber, Ronald K.; Huang, Horng-chih; Toth, Mihaly V.; Moormann, Alan E.; Snyder, Jeffrey S.; Scholten, Jeffrey A.; Garland, Danny J.; Rueppel, Melvin L.; Neumann, William L.; Long, Scott; Wei, Huang; Trujillo, John; Parlow, John J.; Jones, Darin E.; Case, Brenda; Hayes, Michael J.; Zeng, Qingping; Abbas, Zaheer; Fenton, Ricky L.; Kusturin, Carrie L.; Hayat, Rahman K.; Sample, Kirby R.; Schweitzer, Barbara A.; Wood, Rhonda S.; Szalony, Jim; Suleymanov, Osman D.; Salyers, Anita; Nicholson, Nancy S. (Pharmacia Corporation, USA). PCT Int. Appl. WO 2003029224 A1 20030410, 495 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US31784 20021003. PRIORITY: US 2001-PV326721 20011003; US 2001-PV338623 20011024; US 2001-PV332857 20011106; US 2001-PV333292 20011114; US 2001-PV332107 20011121; US 2001-PV332014 20011121.

GI



I



II

AB The present invention relates to compds. (shown as I; variables defined below), and prodrug compds. thereof (e.g. 3-amino-5-[1-[2-[[4-[amino(imino)methyl]benzyl]amino]-2-oxoethyl]-3-bromo-5-(isopropylamino)-6-oxo-1,6-dihydropyrazin-2-yl]benzoic acid (shown as II)), as well as compns. and **methods** useful for preventing and treating thrombotic conditions in mammals. Data showing the selective **inhibition** of tissue factor-VIIa relative to thrombin and human factor Xa are tabulated for >200 examples of I. The effect of 2 examples

of I in combination with aspirin for the treatment of thrombus in mammals are tabulated. Although the **methods** of preparation are not claimed, .apprx.260 example prepns. are included. For I: X5 is CH, C(F), or C(Br); L1 is a linker, linking Z1 to the heterocyclic ring; Z1 is C1-C8 alkyl, C2-C8 alkenyl, or C2-C8 alkynyl, the alkyl, alkenyl, or alkynyl being optionally substituted at any substitutable position with F, hydroxy, carboxy, or alkoxycarbonyl. Z3 comprises a substituted Ph, thienyl, or furanyl ring, the Ph, thienyl or furanyl ring being substituted with an amidine or a derivatized amidine group, and optionally further substituted at any substitutable position with F, hydroxy, carboxy, alkoxycarbonyl, or hydrocarbyloxy. Z4 comprises a 5- or 6-membered heteroaryl or aryl ring. Provided, however, one of the following conditions exist: (a) Z1 is other than unsubstituted cyclobutyl when X5 is CH; (b) Z1 is other than unsubstituted iso-Pr when (i) X5 is CH and (ii) Z4 is 3,5-diaminophenyl or 3-amino-5-(2,2,2-trifluoroacetamide)phenyl; or (c) Z3 is other than 4-amidinobenzyl, 4-amidino-2-fluorobenzyl, or 4-amidino-3-fluorobenzyl.

L24 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN

2003:282399 Document No. 138:304302 Preparation of amidine-substituted polycyclic compound prodrugs useful for selective **inhibition** of serine proteases of the coagulation cascade. South, Michael S.; Webber, Ronald K.; Huang, Horng-chih; Toth, Mihaly V.; Moormann, Alan E.; Snyder, Jeffrey S.; Scholten, Jeffrey A.; Garland, Danny J.; Rueppel, Melvin L.; Neumann, William L.; Long, Scott; Wei, Huang; Trujillo, John; Parlow, John J.; Jones, Darin E.; Case, Brenda; Hayes, Michael J.; Zeng, Qingping (Pharmacia Corporation, USA). PCT Int. Appl. WO 2003028729 A2 20030410, 547 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US31468 20021003. PRIORITY: US 2001-PV326721 20011003; US 2001-PV338623 20011024; US 2001-PV332857 20011106; US 2001-PV350052 20011107; US 2001-PV344957 20011107; US 2001-PV333292 20011114; US 2001-PV332104 20011121; US 2001-PV332014 20011121; US 2001-PV331891 20011121.

GI

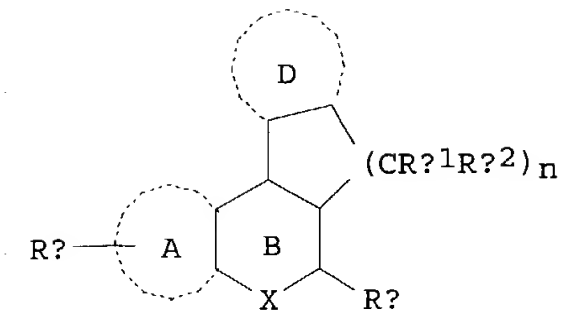
* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY - AVAILABLE VIA OFFLINE PRINT *

AB The present invention relates to prodrug compds., comprising a 5- or 6-membered heterocyclic or aromatic ring substituted with a derivatized amidine (shown as I and II; variables defined below; e.g. N-[4-[(Z)-amino[(pyridin-2-ylmethoxy)imino]methyl]benzyl]-2-[6-[3-amino-5-(trifluoromethyl)phenyl]-3-(isopropylamino)-2-oxopyrazin-1(2H)-yl]acetamide (shown as III)), as well as compns. and **methods** useful for preventing and treating thrombotic conditions in mammals. The prodrug compds. of the present invention selectively **inhibit** certain serine proteases of the coagulation cascade (no data). For I: X = 5- or 6-membered heterocyclic or aromatic ring, the ring atoms being X1, X2, X3, X4, and X5 for 5-membered heterocyclic rings and X1, X2, X3, X4, X5 and X6 for 6-membered heterocyclic or aromatic rings, wherein X2 is alpha to each of X1 and X3, X3 is alpha to each of X2 and X4, X4 is alpha to each of X3 and X5, X5 is alpha to X4 and alpha to X1 if X is a 5-membered ring or to X6 if X is a 6-membered ring, and X6, when present, is alpha to each of X1 and X5, wherein X1, X2, X3, X4, X5 and X6 are C, N, O or S. L1, L3 and L4 are linkages through which Z1, Z3, and Z4, resp., are covalently bonded to different ring atoms of the 5- or 6-membered heterocyclic or aromatic ring of X, wherein Z1 is covalently bonded to X1, Z3 is covalently bonded to X3,

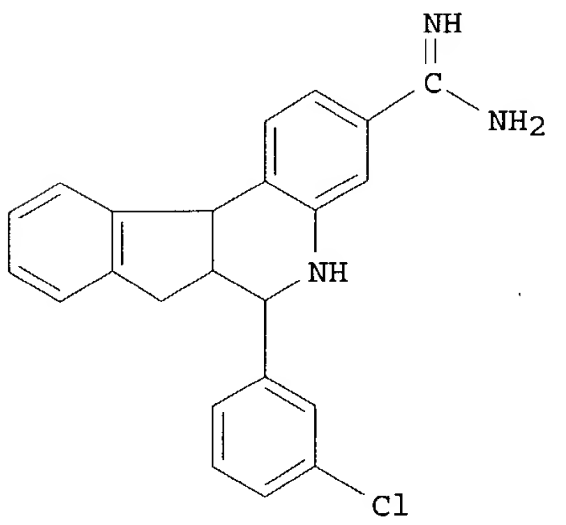
and Z4 is covalently bonded to X4, each of L1, L3 and L4 independently being a covalent bond or comprising ≥1 atoms through which Z1, Z3, and Z4 are covalently bonded to X1, X3 and X4, resp. Z1 is hydrocarbyl or substituted hydrocarbyl; Z3 = 5- or 6-membered heterocyclic or aromatic ring substituted with a derivatized amidine which, upon hydrolysis, oxidation, reduction or elimination yields an amidine group, and optionally further substituted with a halogen or hydroxy, the ring atoms of the 5- or 6-membered heterocyclic or aromatic ring of Z3 being C, S, N, or O. Z4 = 5- or 6-membered heterocyclic or carbocyclic ring having two substituents, R42 and R44, and two ring atoms each of which is in the beta position relative to the ring atom of Z4 through which Z4 is covalently bonded to X, wherein one of R42 and R44 is covalently bonded to one of said beta positions and the other of R42 and R44 is covalently bonded to the other of said beta positions, the ring atoms of the 5- or 6-membered heterocyclic or carbocyclic ring of Z4 being C, N, O, or S. R42 is amino; and R44 = H, hydrocarbyl, substituted hydrocarbyl, heterocyclo, halogen, or a (un)substituted heteroatom = N, O, S and P; provided, however, the derivatized amidine is other than amidine derivatized with tert-butoxycarbonyl. For II: each of X1, X2, X3, X4, X5 and X6 is C or N; X2 is a H bond acceptor; X9 is a direct bond or -(CH2)m- where m is 1 to 5. The metabolic stability and/or bioavailability of .apprx.20 examples of I/II are tabulated. Although the **methods** of preparation are not claimed, .apprx.160 example prepns. are included.

L24 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN
2003:154202 Document No. 138:187653 Preparation of tetracyclic tetrahydroquinoline **inhibitors** of serine proteases as antithrombotic agents. Zhou, Jinglan; Robinson, Leslie; Gubernator, Nikolaus; Saiah, Eddine; Bai, Xu; Gu, Xin (Bristol-Myers Squibb Company, USA). PCT Int. Appl. WO 2003015715 A2 20030227, 311 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US26967 20020820. PRIORITY: US 2001-PV313549 20010820.

GI



I



II

AB This invention relates generally to tetracyclic tetrahydroquinoline compds. (shown as I; variables defined below; e.g. 6-(3-chlorophenyl)-5,6a,7,11b-tetrahydro-6H-indeno[2,1-c]quinoline-3-carboxamidine), and analogs thereof, and pharmaceutically acceptable salt forms thereof, which are selective **inhibitors** of serine protease enzymes, especially factor VIIa; pharmaceutical compns. containing the same; and **methods** of using the same as anticoagulant agents for modulation of the coagulation cascade. Although the **methods** of preparation are not claimed, 240 example prepns. are included. Compds. I demonstrated Ki values of ≤50 μM in assays of **inhibition** of 5 coagulation factors; values for specific I are not given. For I: X is -NH-, -O-, -S-, -S(O)-, or -S(O)2-; ring A, including the two atoms of Ring B to which it is attached, is a Ph ring; wherein, in addition to RA, ring A is substituted with 0-3 RAA; alternatively, ring A, including the two atoms of Ring B to which it is attached, is a 5-6 membered aromatic system consisting of C atoms and 1 or 2 N atoms, and ring A, in addition to RA, is substituted with 0-3 RAA; alternatively ring A and substituent RA, including the two atoms of Ring B to which ring A is attached, is a 5-6 membered heterocyclic ring; alternatively ring A and substituent RA, including the two atoms of Ring B to which Ring A is attached, is a Ph ring wherein RA is combined with RAA and two C atoms of Ring A to form a cyclic group. RA = F, Cl, Br, OH, OCH3, OCH2CH3, OCHMe2, -OCH2CH2CH3, -OCF3, -CN, -NH2, -NH2NH3, C(:NR1)NR2R3, R-NHC(:NR1)NR2R3, -NR2CH(:NR1), -C(O)NR2R3, -S(O)2NR2aR31, -NR2R3, -CH2NR2R3, -CH2CH2NR2R3, -CHMeNR2R3, -CH2CH2CH2NR2R3, -CH2CHMeNR2R3, -CHEtNR2R3, -CHMeCH2NR2R3, -CMe2NR2R3, -(C1-3alkyl)CO2H, -O-(C1-3 alkyl)CO2H, -S-(C1-3 alkyl)CO2H, and -(C1-3 alkyl)CH(NH2)CO2H, -C(O)NHCH2CH2NH(C1-3 alkyl), -C(O)NHCH2CH2N(C1-3 alkyl)2, -CH2NCOO(C1-4 alkyl), imidazol-1-yl, substituted 2,5-dihydro-5-oxopyrazol-3-yl, 4,5-dihydroimidazol-2-ylamino, and 1,4,5,6-tetrahydropyrimidin-2-ylamino. RB is a 5-10 membered ring system consisting of C atoms and 0, 1 or 2 heteroatoms N, O, and S; wherein said ring system may be unsatd., partially unsatd. or saturated; and RB is substituted with 0-5 substituents = Rb1, Rb2, Rb3, Rb4, and Rb5; alternatively RB is C1-4 alkyl substituted with 5-10 membered ring system consisting of C atoms and 0, 1 or 2 heteroatoms N, O, and S; wherein said ring system may be unsatd., partially unsatd. or saturated; and RB is substituted with 0-5 substituents = Rb1, Rb2, Rb3, Rb4, and Rb5. N is 1, 2, or 3; RC1 = H, halo, -CN, -NO2,

OR12, SR12, NR12R13, C(O)H, C(O)R12, C(O)NR12R13, OC(O)NR12R13, NR14C(O)R12, NR14C(S)R12, C(O)OR12, OC(O)R12, OC(O)OR12, CH(:NR14)NR12R13, NHC(:NR14)NR12R13, S(O)R12, S(O)2R12, S(O)NR12R13, S(O)2NR12R13, NR14S(O)R12, NR14S(O)2R12, NR12C(O)R15, NR12C(S)R15, NR12C(O)OR15, NR12S(O)2R15, NR12C(O)NHR15, C1-4 haloalkyl, (C1-4 haloalkyl)oxy, C1-10 alkyl substituted with 0-3 RCC, C2-10 alkenyl substituted with 0-3 RCC, C2-10 alkynyl substituted with 0-3 RCC, C1-10 alkoxy substituted with 0-3 RCC, C3-6 carbocyclic residue substituted with 0-3 RCC, aryl substituted with 0-5 RCC, and 5-6 membered heterocyclic ring system containing = 1-4 heteroatoms N, O, and S substituted with 0-3 RCC; RC2 = H, C1-4 alkyl, OH, CN, and C1-4 alkoxy. Ring D, including the two atoms of Ring C to which it is attached, is a 5-6 membered aromatic system consisting of C atoms and 0, 1 or 2 heteroatoms N, O, and S; and ring D is substituted with 0-4 RD; addnl. details regarding the above variables are given in the claims.

L24 ANSWER 4 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:120325 The Genuine Article (R) Number: 637ZZ. In vitro and in silico design of alpha(1)-antitrypsin mutants with different conformational stabilities. Gilis D; McLennan H R; Dehouck Y; Cabrita L D; Rooman M; Bottomley S P (Reprint). Monash Univ, Dept Biochem & Mol Biol, Struct Biol Grp, Wellington Rd, POB 13D, Clayton, Vic 3800, Australia (Reprint); Monash Univ, Dept Biochem & Mol Biol, Struct Biol Grp, Clayton, Vic 3800, Australia; Free Univ Brussels, B-1050 Brussels, Belgium. JOURNAL OF MOLECULAR BIOLOGY (17 JAN 2003) Vol. 325, No. 3, pp. 581-589. Publisher: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD. 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND. ISSN: 0022-2836. Pub. country: Australia; Belgium. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB alpha(1)-Antitrypsin, a protein belonging to the **serine protease inhibitor** (serpin) superfamily, is characterized by the ability to undergo dramatic conformational changes leading to inactive polymers. Serpin polymerization, which causes a range of diseases such as emphysema, thrombosis and dementia, occurs through a process in which the reactive center loop residues of one serpin molecule insert into the A beta-sheet of another. PoP-MuSiC, a program that uses database-derived mean force potentials to predict changes in folding free energy resulting from single-site mutations, was used to modulate rationally the polymerization propensity of alpha(1)-antitrypsin. This was accomplished by generating mutants with a stabilized active form and destabilized polymerized form, or the converse. Of these mutants, five were expressed and characterized experimentally. In agreement with the predictions, three of them, K331F, K331I and K331V, were shown to stabilize the active form and decrease the polymerization rate, and one of them, S330R, to destabilize the active form and to increase polymerization. Only one mutant (K331T) did not display the expected behavior. Thus, strikingly, the adjacent positions 330 and 331, which are located at the beginning of the beta-strand next to the additionally inserted beta-strand in the polymerized form, have opposite effects on the conformational change. These residues therefore appear to play a key role in inducing or preventing such conformational change.

L24 ANSWER 5 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:445879 Document No.: PREV200200445879. Savignygrin, a platelet **aggregation inhibitor** from the soft tick Ornithodoros savignyi, presents the RGD integrin recognition motif on the Kunitz-BPTI fold. Mans, Ben J.; Louw, Abraham I.; Neitz, Albert W. H. [Reprint author]. Department of Biochemistry, University of Pretoria, Pretoria, 0002, South Africa. albert.neitz@bioagric.up.ac.za. Journal of Biological Chemistry, (June 14, 2002) Vol. 277, No. 24, pp. 21371-21378. print. CODEN: JBCHA3. ISSN: 0021-9258. Language: English.
AB Savignygrin, a platelet **aggregation inhibitor** that possesses the RGD integrin recognition motif, has been purified from the soft tick Ornithodoros savignyi. Two isoforms with similar biological activities differ because of R52G and N60G in their amino acid sequences,

indicating a recent gene duplication event. Platelet **aggregation** induced by ADP (IC50, 130 nM), collagen, the thrombin receptor-activating peptide, and epinephrine was **inhibited**, although platelets were activated and underwent a shape change. The binding of alpha-CD41 (P2) to platelets, the binding of purified alphaIIbbeta3 to fibrinogen, and the adhesion of platelets to fibrinogen was **inhibited**, indicating a targeting of the fibrinogen receptor. In contrast, the adhesion of osteosarcoma cells that express the integrin alphavbeta3 to vitronectin or fibrinogen was not **inhibited**, indicating the specificity of savignygrin toward alphaIIbbeta3. Savignygrin shows sequence identity to disagregin, a platelet **aggregation inhibitor** from the tick Ornithodoros moubata that lacks an RGD motif. The cysteine arrangement of savignygrin is similar to that of the bovine pancreatic trypsin **inhibitor** family of **serine protease inhibitors.** A homology model based on the structure of the tick anticoagulant peptide indicates that the RGD motif is presented on the substrate-binding loop of the canonical BPTI **inhibitors.** However, savignygrin did not **inhibit** the serine proteases fXa, plasmin, thrombin, or trypsin. This is the first report of a platelet **aggregation inhibitor** that presents the RGD motif using the Kunitz-BPTI protein fold.

L24 ANSWER 6 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2002:535685 The Genuine Article (R) Number: 567YJ. Association between conformational mutations in neuroserpin and onset and severity of dementia . Davis R L; Shrimpton A E; Carrell R W (Reprint); Lomas D A; Gerhard L; Baumann B; Lawrence D A; Yepes M; Kim T S; Ghetti B; Piccardo P; Takao M; Lacbawan F; Muenke M; Sifers R N; Bradshaw C B; Kent P F; Collins G H; Larocca D; Holohan P D. Univ Cambridge, Dept Haematol, Cambridge Inst Med Res, Cambridge, England (Reprint); Univ Cambridge, Dept Med, Cambridge Inst Med Res, Cambridge CB2 2QQ, England; Upstate Med Univ, Dept Pathol, Syracuse, NY USA; Univ Witten Herdecke, Inst Clin Neurosurg, Witten, Germany; Univ Magdeburg, Dept Psychiat, D-39106 Magdeburg, Germany; Amer Red Cross, Holland Lab, Dept Vasc Biol, Rockville, MD USA; Georgetown Univ, Med Ctr, Dept Neurol, Washington, DC 20007 USA; Yonsei Univ, Coll Med, Dept Pathol, Seoul, South Korea; Indiana Univ, Sch Med, Div Neuropathol, Alzheimer Dis Ctr, Indianapolis, IN USA; Childrens Natl Med Ctr, Dept Med Genet, Washington, DC 20010 USA; NHGRI, Med Genet Branch, NIH, Bethesda, MD 20892 USA; Baylor Coll Med, Dept Pathol, Houston, TX 77030 USA; Upstate Med Univ, Dept Neurol, Syracuse, NY USA; Upstate Med Univ, Dept Pharmacol, Syracuse, NY USA. LANCET (29 JUN 2002) Vol. 359, No. 9325, pp. 2242-2247. Publisher: LANCET LTD. 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND. ISSN: 0140-6736. Pub. country: England; USA; Germany; South Korea. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background The **aggregation** of specific proteins is a common feature of the familial dementias, but whether the formation of neuronal inclusion bodies is a causative or incidental factor in the disease is not known. To clarify this issue, we investigated five families with typical neuroserpin inclusion bodies but with various neurological manifestations.

Methods Five families with neurodegenerative disease and typical neuronal inclusions had biopsy or autopsy material available for further examination. Immunostaining confirmed that the inclusions were formed of neuroserpin aggregates, and the responsible mutations in neuroserpin were identified by sequencing of the neuroserpin gene (SERPINI1) in DNA from blood samples or from extraction of histology specimens. Molecular modelling techniques were used to predict the effect of the gene mutations on three-dimensional protein structure. Brain sections were stained and the topographic distribution of the neuroserpin inclusions plotted.

Findings Each of the families was heterozygous for an aminoacid substitution that affected the conformational stability of neuroserpin. The least disruptive of these mutations (S49P), as predicted by molecular modelling, resulted in dementia after age 45 years, and presence of neuroserpin inclusions in only a few neurons. By contrast, the most

severely disruptive mutation (G392E) resulted, at age 13 years, in progressive myoclonus epilepsy, with many inclusions present in almost all neurons.

Interpretation The findings provide evidence that inclusion-body formation is in itself a sufficient cause of neurodegeneration, and that the onset and severity of the disease is associated with the rate and magnitude of neuronal protein **aggregation.**

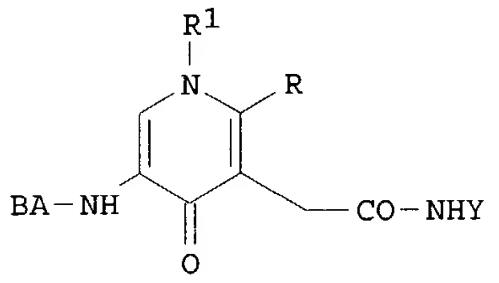
L24 ANSWER 7 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:90484 Document No.: PREV200300090484. Aprotinin Reduces Blood Loss in Pediatric Craniofacial Surgery. Munro, Hamish M. [Reprint Author]; Tait, Alan R. [Reprint Author]; Stricker, Lori J. [Reprint Author]; Muraszko, Karin M. [Reprint Author]; Buchman, Steven R. [Reprint Author]. Section of Pediatric Anesthesiology, University of Michigan, Ann Arbor, MI, USA. Anesthesiology Abstracts of Scientific Papers Annual Meeting, (2002) No. 2000, pp. Abstract No. 1290. http://www.asa-abstracts.com. cd-rom. Meeting Info.: 2000 Annual Meeting of the American Society of Anesthesiologists. San Francisco, CA, USA. October 16-18, 2000. American Society of Anesthesiologists Inc.
Language: English.

AB Introduction: Aprotinin, a **serine protease inhibitor**, exerts multiple effects on coagulation resulting in an **inhibition** of fibrinolysis and platelet **aggregation**, and a reduction in blood loss. It has been used in pediatric open heart surgery and lung transplantation and has been shown to reduce blood loss and transfusion requirements. Reconstructive craniofacial surgery has the potential for large intraoperative blood loss yet the use of aprotinin in this population has not been reported. Therefore, a pilot study was undertaken to ascertain whether aprotinin would reduce blood loss and transfusion requirements for this type of surgery. **Methods**: Ten consecutive children <6 years of age, undergoing reconstructive craniofacial surgery were prospectively assigned to receive aprotinin (Group A) intraoperatively in an unblinded fashion. Following induction of general anesthesia, all children received a 1ml (1.4mg) test dose of aprotinin, a loading dose of 240 mg/m2 and a continuous infusion of 56 mg/m2/hr for the duration of surgery. Outcome data included estimated blood loss (suction containers and surgical sponges), blood product administration, length of surgery and hospital stay, and postoperative transfusion requirements. Two groups were used for comparison, a control group (Group C) which included 10 consecutive children having similar surgery immediately prior to the study, and a matched set (Group M) identified from records and matched for procedure, age and weight. Data were analyzed using ANOVA with post hoc pairwise comparisons. $P < 0.05$ was considered significant. Results: Thirty children (mean age 18 months, mean weight 10.7 kgs) were studied. There were no differences between groups with regards to age, weight, starting and postoperative Hct, or length of surgery. The mean blood loss in Group A (247 mls) was under half that of groups C (550 mls, $p<0.001$) and M (540 mls, $p=0.08$). Similarly, red cell transfusion was lower in group A compared to Groups C and M (144 vs 475 mls, $p<0.001$ and 352 mls, $p=0.001$ respectively). All patients in Groups C and M required intraoperative blood transfusion compared with only 5/10 (50%) in Group A ($p<0.05$). In the ICU, one further patient in the aprotinin group received blood products compared to four in the control group and three in the matched group. There were no differences between groups with respect to length of ICU or hospital stay. Discussion: While this preliminary report is limited by it's retrospective and non-randomized design, interim results show that intraoperative blood loss was substantially reduced with the use of aprotinin, resulting in an reduced need for transfusion and therefore exposure to multiple blood products. A randomized prospective double-blind trial will determine whether aprotinin truly has a place in pediatric craniofacial surgery.

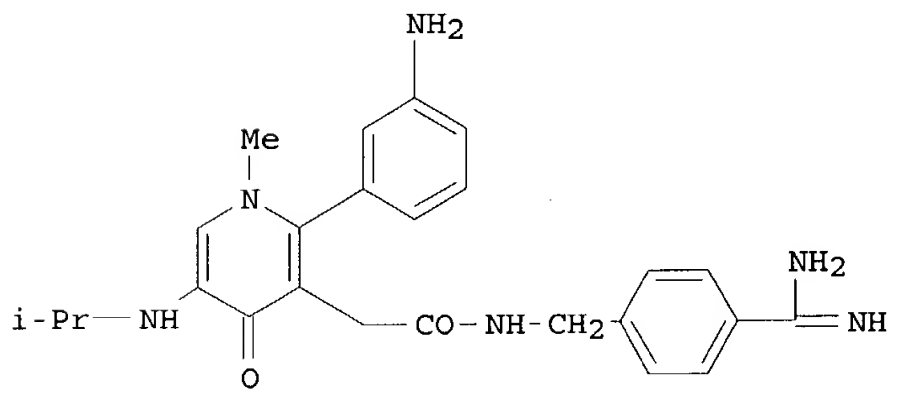
L24 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN
2001:762971 Document No. 135:303781 Preparation of polycyclic aryl and heteroaryl substituted 4-pyridones useful for selective **inhibition**

of the coagulation cascade. South, Michael S.; Ma, Chun C.; Koeller, Kevin J.; Rahman, Hayat K.; Neumann, William L. (Pharmacia Corporation, USA). PCT Int. Appl. WO 2001077079 A2 20011018, 431 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11014 20010404. PRIORITY: US 2000-PV194851 20000405; US 2000-PV252031 20001120.

GI



I



II

AB Title compds. [I; R = 3-NH2C6H4, 3-NH2CO-5-NH2C6H3, 3-NH2-5-(2-ClC6H4CH2NHCO)C6H3, 3-NH2-5-(2-CF3C6H4CH2NHCO)C6H2, 3-NH2-5-(2-ClC6H4CH2NHSO2)C6H3, 5-NH2-2-FC6H3, 2-CH3-3-NH2C6H3, 3-NH2-5-HOOCC6H3, 3,5-(NH2)2C6H3, 3-NH2-5-C6H5CH2NHCOC6H3; R1 = CH3, CH3(CH2)2, H; BA = C6H5CH2, 3-ClC6H4CH2CH2, 3-(2-imidazoyl)propyl, CF3CH2, CH2:CHCH2, CH3CH:CHCH2, (R)-(CH3CH2)(CH3)CH, CHCCH2, CH3, (CH3)3C, CF3CH2, (S)-(CH3CH2)(CH3)CH, (CH3)2CH, CH3CH2, CH3(CH2)2, NH2(CH2)6, HO(CH2)3; Y = 4-(NH2)(NH)CC6H4CH2, 2-F-4-(NH2)(NH)CC6H3CH2, 3-F-4-(NH2)(NH)CC6H3CH2, etc.] and pharmaceutically acceptable salts are prepared as **serine proteases inhibitors** in mammal. **Methods** for anticoagulant therapy for the treatment and prevention of a variety of thrombotic conditions including coronary artery and cerebrovascular diseases using title compds. I are related. Thus,the title compound II was prepared and biol. activity tested.

L24 ANSWER 9 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN
2001:228928 Document No. 134:247248 Bivalent **inhibitor** of FVIIa/tissue factor/FXa complex and therapeutic use. Freskgaard, Per-Ola; Jakobsen, Palle (Novo Nordisk A/S, Den.). PCT Int. Appl. WO 2001021661 A1 20010329, 55 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,

YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-DK516 20000919. PRIORITY: DK 1999-1333 19990920; US 1999-PV159773 19991015.

AB A bivalent **serine protease inhibitor** of coagulation factor VIIa and factor Xa is provided which comprises: (i) a first **serine protease inhibitor** binding to factor VIIa; (ii) a linker moiety; and (iii) a second **serine protease inhibitor** binding to factor Xa. Also provided are a **method** for **inhibiting** the two different serine proteases factor VIIa and factor Xa simultaneously and selectively when the two serine proteases becomes localized on the membrane protein tissue factor (TF). The compds. and **method** are useful for prevention or treatment of FVIIa/TF-related diseases or disorders, e.g. deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplasty (PTCA), stroke, tumor metastasis, inflammation, septic chock, hypotension, ARDS, pulmonary embolism, disseminated intravascular coagulation (DIC), vascular restenosis, platelet deposition, myocardial infarction, angiogenesis, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis. Preparation of e.g. octanedioic acid bis-[(1-(1-(1-chloroacetyl-4-guanidinobutylcarbamoyl)2-phenylethylcarbamoyl)2-phenylethyl)amide] is described.

L24 ANSWER 10 OF 27 MEDLINE on STN

2001448130. PubMed ID: 11327057. Thrombin-stimulated growth, clustering, and collagen lattice contraction of human gingival fibroblasts is associated with its protease activity. Chang M C; Chan C P; Wu H L; Chen R S; Lan W H; Chen Y J; Jeng J H. (Team of Biomedical Science, Chang-Gung Institute of Nursing, Taoyuan, Taiwan.) Journal of periodontology, (2001 Mar) 72 (3) 303-13. Journal code: 8000345. ISSN: 0022-3492. Pub. country: United States. Language: English.

AB BACKGROUND: Thrombin is a serine protease produced following gingival tissue injury or inflammation. It regulates the functional behavior of injury-neighboring cells via the activation of specific protease-activated receptors (PAR). Thrombin's role in gingival tissue healing and inflammatory response processes is not yet well understood. **METHODS**: We investigated the effects of thrombin on gingival fibroblast (GF) growth [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay], collagen lattice contraction, and associated morphological changes. RESULTS: Thrombin (>1 U/ml), but not thrombin receptor (PAR-1) agonist peptide (SFLLRN, single letter amino acid code, abbreviated as TRAP, 1 to 50 microg/ml), stimulated the growth and clustering of cultured human GF in vitro. Growth-stimulatory effects of thrombin were **inhibited** by D-Phe-Pro-ArgCH2Cl (PPACK), a **serine protease inhibitor.** By contrast, trypsin (>10 microg/ml), a PAR-2 activator, suppressed the growth of GF. Thrombin (>0.2 U/ml) and TRAP (10 to 25 microg/ml), but not trypsin, prostaglandin E2 (0.01 to 0.5 microg/ml), or bovine serum albumin (BSA) (1 to 80 microg/ml), induced the GF-populated collagen lattice contraction within 30 to 60 minutes of exposure. The thrombin-induced collagen lattice contraction was **inhibited** by PPACK (20 microg/ml) and an actin filament polymerization **inhibitor**, cytochalasin B (1 microg/ml). The collagen lattice contraction induced by TRAP was also **inhibited** by cytochalasin B, but not by PPACK. Using a reverse-transcriptase polymerase chain reaction (RT-PCR), the expression of PAR-1, and to a lesser extent PAR-3, was observed for human GF, although little PAR-2 and PAR-4 expression was noted. CONCLUSIONS: These results indicate that thrombin is important in periodontal wound healing and inflammatory processes by promoting the growth and contraction of GF. The stimulatory effects of thrombin are associated with its protease activation of thrombin receptors.

L24 ANSWER 11 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

2002:176600 Document No.: PREV200200176600. A novel metalloprotease secreted by Escherichia coli O157:H7 cleaves C1 esterase **inhibitor**, a regulator of multiple proteolytic cascades. Lathem, W. W. [Reprint author]; Welch, R. A. [Reprint author]. University of Wisconsin, Madison, WI, USA. Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 113. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011. Language: English.

AB Enterohemorrhagic Escherichia coli (EHEC) are responsible for diarrheal disease, hemorrhagic colitis, and hemolytic uremic syndrome that can lead to acute renal failure and death. Strains of the serotype O157:H7 carry a large virulence plasmid designated pO157 that encodes genes for multiple virulence factors. We have identified a gene on pO157 of previous unknown function whose product causes the serum-dependent **aggregation** of two cultured human CD4+ T cell lines, Jurkat and MOLT-4, but not a B cell lymphoma line (Raji), or macrophage-like cell lines (U937 and HL-60). The protein, named StcE for secreted T cell **aggregation** factor from EHEC, contains a putative N-terminal signal sequence and lies immediately upstream of the etp type II protein secretion cluster of pO157. A recombinant form of StcE (StcE-His) interacts with a human serum protein(s) of approximately 105 kDa as determined by Far Western blotting analysis. This protein was identified by mass spectrometry as plasma C1 esterase **inhibitor** (C1INH). C1INH is a regulatory protein responsible for controlling several proteolytic cascades, including the classical complement pathway. StcE-His specifically cleaves purified C1INH to produce an approximately 60 kDa fragment in a zinc-dependent manner; StcE-His also acts on C1INH in human serum. Additionally, bacterial culture supernatants containing native StcE cleave C1INH as described. StcE may represent a new class of bacterial virulence factors termed inserpins (**inhibitors** of **serine protease inhibitors**) which act to disregulate the host's ability to control the inappropriate activation of the complement, kallikrein, and coagulation cascades. This may result in an unregulated pro-inflammatory and coagulation response that may be responsible for tissue damage in the intestine and kidney in patients infected with enterohemorrhagic strains of E. coli.

L24 ANSWER 12 OF 27 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

2000:931757 The Genuine Article (R) Number: 379XK. Astroglial expression of human alpha(1)-antichymotrypsin enhances Alzheimer-like pathology in amyloid protein precursor transgenic mice. Mucke L (Reprint); Yu G Q; McConlogue L; Rockenstein E M; Abraham C R; Masliah E. UNIV CALIF SAN FRANCISCO, GLADSTONE INST NEUROL DIS, POB 41900, SAN FRANCISCO, CA 94141 (Reprint); UNIV CALIF SAN FRANCISCO, DEPT NEUROL, SAN FRANCISCO, CA 94141; UNIV CALIF SAN FRANCISCO, NEUROSCI PROGRAM, SAN FRANCISCO, CA 94141; ELAN PHARMACEUT, S SAN FRANCISCO, CA; UNIV CALIF SAN DIEGO, DEPT NEUROSCI, LA JOLLA, CA 92093; UNIV CALIF SAN DIEGO, DEPT PATHOL, LA JOLLA, CA 92093; BOSTON UNIV, SCH MED, DEPT BIOCHEM, BOSTON, MA 02118; BOSTON UNIV, SCH MED, DEPT MED, BOSTON, MA 02118. AMERICAN JOURNAL OF PATHOLOGY (DEC 2000) Vol. 157, No. 6, pp. 2003-2010. Publisher: AMER SOC INVESTIGATIVE PATHOLOGY, INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3993. ISSN: 0002-9440. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Proteases and their inhibitors play key roles in physiological and pathological processes. Cerebral amyloid plaques are a pathological hallmark of Alzheimer's disease (AD). They contain amyloid-beta (A beta) peptides in tight association with the **serine protease inhibitor** alpha (1)-antichymotrypsin.(1,2) However, it is unknown whether the increased expression of alpha (1)-antichymotrypsin found in AD brains counteracts or contributes to the disease. We used regulatory sequences of the glial fibrillary acidic protein gene(3) to express human alpha (1)-antichymotrypsin (hACT) in astrocytes of transgenic mice. These mice were crossed with transgenic mice that produce human amyloid protein

precursors (hAPP) and A beta in neurons.(4,5) No amyloid plaques were found in transgenic mice expressing hACT alone, whereas hAPP transgenic mice and hAPP/ hACT doubly transgenic mice developed typical AD-like amyloid plaques in the hippocampus and neocortex around 6 to 8 months of age. Co-expression of hAPP and hACT significantly increased the plaque burden at 7 to 8, 14, and 20 months. Both hAPP and hAPP/hACT mice showed significant decreases in synaptophysin-immunoreactive presynaptic terminals in the dentate gyrus, compared with nontransgenic littermates. Our results demonstrate that hACT acts as an amyloidogenic co-factor in vivo and suggest that the role of hACT in AD is pathogenic.

L24 ANSWER 13 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2000:110924 Document No.: PREV200000110924. Human placental trophoblasts secrete a disintegrin metalloproteinase very similar to the insulin-like growth factor binding protein-3 protease in human pregnancy serum. Irwin, Juan C.; Suen, Lii-Fang; Cheng, Bi-Hua; Martin, Robert; Cannon, Paul; Deal, Cheri L.; Giudice, Linda C. [Reprint author]. Department of Gynecology and Obstetrics, Stanford University Medical Center, Stanford, CA, 94305-5317, USA. Endocrinology, (Feb., 2000) Vol. 141, No. 2, pp. 666-674. print.
CODEN: ENDOAO. ISSN: 0013-7227. Language: English.

AB During the course of human pregnancy, there is a marked increase in insulin-like growth factor (IGF) binding protein (IGFBP)-3 protease activity in maternal serum that is first evident at 6 weeks of gestation, persists through term, and returns to nonpregnancy levels by day 5 postpartum. This protease activity cleaves IGFBP-3 into smaller fragments that have markedly reduced affinity for the IGFs. To date, the precise identity and cellular origin of the pregnancy-associated serum IGFBP-3 protease have not been established. To investigate whether placental and/or decidual tissues, which uniquely develop during pregnancy, may be sources of the pregnancy-associated serum IGFBP protease, we examined the secretion of IGFBP-3 protease in vitro by isolated human cytotrophoblasts or fibroblasts from second trimester placentae and by in vitro decidualized human endometrial stromal cells. Cytotrophoblasts were either cultured alone, which favors **aggregation** and fusion, or cocultured with decidualized endometrial stromal cells, which favors differentiation to an invasive phenotype. IGFBP-3 protease activity was detected in trophoblast, but not in placental fibroblast or decidualized endometrial cultures, and was also present in trophoblast-endometrial cocultures. Western ligand blot and Western immunoblot analyses showed that most of the endogenous IGFBP-3 in trophoblast cultures was in the form of low molecular weight fragments with reduced IGF binding affinity. The substrate specificity of the trophoblast-derived protease was identical to that in pregnancy serum, showing activity against IGFBP-2, -3, and -4, but being inactive against IGFBP-1. IGFBP-3 proteolysis by both pregnancy serum and trophoblast conditioned medium showed a major peak of activity at neutral pH. The trophoblast-derived activity caused time- and temperature-dependent proteolysis of IGFBP-3 into fragments of identical size as those produced by pregnancy serum, and also shared its sensitivity to **protease inhibitors**: highly sensitive to EDTA and o-phenanthroline, partially sensitive to the **serine protease inhibitors** AEBSF and aprotinin, and insensitive to alpha2-antiplasmin, and to aspartic and cysteine **protease inhibitors.** IGFBP-3 proteolysis by both pregnancy serum and trophoblast conditioned medium was also insensitive to tissue **inhibitor** of metalloproteinase-1, precluding the involvement of the matrix metalloproteinases. In contrast, both the pregnancy serum- and trophoblast-derived proteases were preferentially **inhibited** by a hydroxamic acid derivative with selective activity against the disintegrin-metalloproteinase tumor necrosis factor-alpha converting enzyme. This study shows that placental trophoblasts produce an IGFBP-3 protease with characteristics very similar to the activity found in pregnancy serum and indicates these cells at the maternal-fetal interface are a potential source of the pregnancy-associated serum IGFBP-3 protease. The findings further suggest that the main IGFBP-3 protease activity in

both pregnancy serum and trophoblast conditioned medium may correspond to a disintegrin-metalloproteinase type enzyme.

L24 ANSWER 14 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:519126 Document No.: PREV199900519126. Modification of liposomes with N-substituted polyacrylamides: Identification of proteins adsorbed from plasma. Yamazaki, A.; Winnik, F. M.; Cornelius, R. M.; Brash, J. L. [Reprint author]. Department of Chemical Engineering, McMaster University, Hamilton, ON, L8S 4L7, Canada. Biochimica et Biophysica Acta, (Sept. 21, 1999) Vol. 1421, No. 1, pp. 103-115. print.
CODEN: BBACAQ. ISSN: 0006-3002. Language: English.

AB Liposomes prepared from DMPC (80%) and cholesterol (20%) were modified with a series of hydrophobically modified N-substituted polyacrylamides, namely, poly(N-isopropylacrylamide) (PNIPAM), poly(N,N-bis(2-methoxyethyl) acrylamide) (PMEAM), and poly((3-methoxypropyl)acrylamide) (PMPAM). The hydrophobic group, N-(4-(1-pyrenylbutyl)-N-n-octadecylamine was attached to one end of the polymer chains to serve as an anchor for incorporation into the liposome bilayer. Liposome-polymer interactions were confirmed using fluorescence spectroscopy and chemical analysis. Microscopy revealed differences in **aggregation** tendency between unmodified and polymer-modified liposomes. Proteins adsorbed to liposome surfaces during exposure to human plasma were identified by immunoblot analysis. It was found that both unmodified and polymer-modified liposomes adsorb a wide variety of plasma proteins. Contact phase coagulation proteins, complement proteins, cell-adhesive proteins, **serine protease inhibitors**, plasminogen, antithrombin III, prothrombin, transferrin, alpha2-microglobulin, hemoglobin, haptoglobin and beta-lipoprotein as well as the major plasma proteins were all detected. Some differences were found between the unmodified and polymer-modified liposomes. The unmodified liposomes adsorbed plasminogen mainly as the intact protein, whereas on the modified liposomes plasminogen was present in degraded form. Also, the liposomes modified with PNIPAM in its extended conformation (below the lower critical solution temperature) appeared to adsorb less protein than those containing the 'collapsed' form of PNIPAM (above the LCST).

L24 ANSWER 15 OF 27 MEDLINE on STN DUPLICATE 1
1999279167. PubMed ID: 10349550. Elevated temperature treatment as a novel **method** for decreasing p57 on the cell surface of Renibacterium salmoninarum. Piganelli J D; Wiens G D; Kaattari S L. (Department of Microbiology, Oregon State University, Corvallis 97331, USA.) Diseases of aquatic organisms, (1999 Apr 15) 36 (1) 29-35. Journal code: 8807037. ISSN: 0177-5103. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Renibacterium salmoninarum is a Gram-positive diplo-bacillus and the causative agent of bacterial kidney disease, a prevalent disease of salmonid fish. Virulent isolates of R. salmoninarum have a hydrophobic cell surface and express the 57-58 kDa protein (p57). Here we have investigated parameters which effect cell hydrophobicity and p57 degradation. Incubation of R. salmoninarum cells at 37 degrees C for > 4 h decreased cell surface hydrophobicity as measured by the salt **aggregation** assay, and decreased the amount of cell associated p57. Incubation of cells at lower temperatures (22, 17, 4 or -20 degrees C) for up to 16 h did not reduce hydrophobicity or the amount of cell associated p57. Both the loss of cell surface hydrophobicity and the degradation of p57 were **inhibited** by pre-incubation with the **serine protease inhibitor** phenylmethylsulfonyl fluoride (PMSF). Cell surface hydrophobicity was specifically reconstituted by incubation with extracellular protein (ECP) concentrated from culture supernatant and was correlated with the reassociation of p57 onto the bacterial cell surface as determined by western blot and total protein stain analyses. The ability of p57 to reassociate suggests that the bacterial cell surface is not irreversibly modified by the 37 degrees C treatment and that p57 contributes to the hydrophobic nature of R. salmoninarum. In summary, we describe parameters effecting the removal of

the p57 virulence factor and suggest the utility of this modification for generating a whole cell vaccine against bacterial kidney disease.

L24 ANSWER 16 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:61554 Document No.: PREV199900061554. Platelets and cardiopulmonary bypass. Hyde, Jonathan A. J. [Reprint author]; Chinn, Joseph A.; Graham, Timothy R.. 65 Metcley Lane, Harbone, Birmingham B17 0HT, UK. Perfusion (London), (Nov., 1998) Vol. 13, No. 6, pp. 389-407. print.
ISSN: 0267-6591. Language: English.

L24 ANSWER 17 OF 27 MEDLINE on STN DUPLICATE 2
1998223072. PubMed ID: 9563608. Prolonged discordant xenograft survival by **inhibition** of the intrinsic coagulation pathway in complement C6-deficient recipients. Jakobs F M; Davis E A; White T; Sanfilippo F; Baldwin W M 3rd. (Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205-2196, USA.) Journal of heart and lung transplantation : official publication of the International Society for Heart Transplantation, (1998 Mar) 17 (3) 306-11. Journal code: 9102703. ISSN: 1053-2498. Pub. country: United States. Language: English.
AB BACKGROUND: Xenotransplantation of vascularized organs between unmodified discordant species results in hyperacute graft rejection within minutes to hours after graft reperfusion. This process is due to the presence of natural xenoreactive antibodies and complement activation, which lead to vessel injury, thrombosis, and hemorrhage. Because multiple components of the coagulation and complement cascades interact with each other, we have investigated the effects of **inhibiting** these systems together. The recombinant Kunitz type **serine protease inhibitor** (KPI-BG022) tested in these experiments **inhibits** factor XIIa, kallikrein, and plasmin. **METHODS**: Cardiac xenografts from male Hartley guinea pigs were heterotopically grafted into male PVG rats that were either sufficient (C6[+]) or deficient (C6[-]) for the complement component C6 and thus formation of the membrane attack complex. Experimental animals received KPI 5 mg/kg intravenously before reperfusion, and control animals received saline placebo. RESULTS: C6(+) recipients rejected their grafts hyperacutely, without a significant difference between KPI-treated (0.12+/-0.05 hours) and placebo-treated (0.13+/-0.06 hours) recipients (n = 10). As expected, C6(-) recipients showed prolonged graft survival (17.65+/-3.45 hours, n = 5). However, a single intravenous bolus of KPI before releasing the clamps further delayed graft rejection in C6(-) recipients (46.2+/-3.3 hours; n = 5). Histologic examination at 2, 6, and 12 hours after transplantation showed platelet **aggregation** and inflammatory infiltrates were significantly decreased in KPI-treated (C6[-]) recipients. However, intragraft hemorrhage was apparent at 6 and 12 hours. CONCLUSIONS: We conclude that in vivo **inhibition** of the intrinsic clotting cascade by functional inactivation of factor XIIa has a synergistic effect with **inhibition** of membrane attack complex formation in preventing hyperacute discordant xenograft rejection.

L24 ANSWER 18 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1997:106848 Document No.: PREV199799406051. Preoperative platelet dysfunction increases the benefit of aprotinin in cardiopulmonary bypass. Ray, Michael J.; Marsh, Neville A.; Just, Sarah J. E.; Perrin, Emma J.; O'Brien, Mark F.; Hawson, Geoffrey A. T.. Dep. Haematol., Prince Charles Hosp., Brisbane, Queensland 4032, Australia. Annals of Thoracic Surgery, (1997) Vol. 63, No. 1, pp. 57-63.
ISSN: 0003-4975. Language: English.
AB Background. This study was designed to determine the benefit of aprotinin therapy in reducing bleeding during and after cardiopulmonary bypass in patients with preoperative platelet dysfunction. Platelet function involvement in the mechanism by which aprotinin acts was also investigated. **Methods.** In a double-blind, randomized study, patients received high-dose aprotinin (n = 54) or placebo (n = 52). Whole blood **aggregation** was measured preoperatively. Platelet function and activation in both groups were assessed intraoperatively and

postoperatively at five times. Results. Aprotinin significantly reduced perioperative bleeding and postoperative blood transfusion. Placebo-treated patients with reduced preoperative platelet **aggregation** bled more postoperatively, but aprotinin reduced the bleeding in patients with normal or reduced platelet function to similar levels. Any cardiopulmonary bypass-induced changes in platelet **aggregation**, platelet activation as measured by P-selectin expression, and von Willebrand factor antigen and function were similar in aprotinin-treated and placebo-treated groups. Conclusions. The mechanism by which aprotinin reduced bleeding was independent of any effect on platelet function. However, aprotinin produced a greater reduction in bleeding among patients whose condition was hemostatically compromised by preoperative platelet dysfunction.aprotinin, platelet **aggregation** , platelet activation, cardiopulmonary bypass, von Willebrand factor.

L24 ANSWER 19 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1996:406919 Document No.: PREV199699129275. The serpin-enzyme complex receptor recognizes soluble, nontoxic amyloid-beta peptide but not aggregated, cytotoxic amyloid-beta peptide. Boland, Kimberly; Behrens, Maria; Choi, Dennis; Manias, Karen; Perlmutter, David H. [Reprint author]. Dept. Pediatrics, Washington Univ. Sch. Med., One Children's Place, St. Louis, MO 63110, USA. Journal of Biological Chemistry, (1996) Vol. 271, No. 30, pp. 18032-18044.
CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

AB There is now extensive evidence that amyloid-beta peptide is toxic to neurons and that its cytotoxic effects can be attributed to a domain corresponding to amyloid-beta 25-35, GSNKGAIIGLM. We have shown recently that the serine proteinase **inhibitor** (serpin)-enzyme complex receptor (SEC-R), a receptor initially identified for binding of alpha-1-antitrypsin (alpha-1-AT) and other **serine protease inhibitors**, also recognizes the amyloid-beta 25-35 domain. In fact, by recognizing the amyloid-beta 25-35 domain, SEC-R mediates cell surface binding, internalization, and degradation of soluble amyloid-beta peptide. In this study, we examined the possibility that SEC-R mediates the neurotoxic effect of amyloid-beta peptide. A series of peptides based on the sequences of amyloid-beta peptide and alpha-1-AT was prepared soluble in dimethyl sulfoxide or insoluble in water and examined in assays for SEC-R binding, for cytotoxicity in neuronal PC12 cells and murine cortical neurons in primary culture, and for **aggregation** in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)analysis. The results show that amyloid-beta peptide 25-35 and amyloid-beta peptide 1-40 prepared soluble in dimethyl sulfoxide compete for binding to SEC-R, are nontoxic, and migrate as monomers in SDS-PAGE analysis. In contrast, the same peptides aged in water did not compete for binding to SEC-R but were toxic and migrated an aggregates in SDS-PAGE. An all-D-amyloid-beta 25-35 peptide was not recognized at all by SEC-R but retained full toxic-aggregating properties. Using a series of deleted, substituted, and chimeric am-beta/alpha-1-AT peptides, toxicity correlated well with **aggregation** but poorly with SEC-R recognition. In a subclone of PC12 cells which developed resistance to the toxic effect of aggregated amyloid-beta 25-35 there was a 2.5-3-fold increase in the number of SEC-R molecules/cell compared with the parent PC12 cell line. These data show that SEC-R does not mediate the cytotoxic effect of aggregated amyloid-beta peptide. Rather, SEC-R could play a protective role by mediating clearance and catabolism of soluble, monomeric amyloid-beta peptide, if soluble amyloid-beta peptide proves to be an in vivo precursor of the insoluble, toxic peptide.

L24 ANSWER 20 OF 27 MEDLINE on STN DUPLICATE 3
97048846. PubMed ID: 8893576. Nafamostat mesilate reduces blood-foreign surface reactions similar to biocompatible materials. Usui A; Hiroura M; Kawamura M; Hibi M; Yoshida K; Murakami F; Tomita Y; Ooshima H; Murase M. (Division of Cardiac Surgery, Owari Prefectural Hospital, Aichi, Japan.) Annals of thoracic surgery, (1996 Nov) 62 (5) 1404-11. Journal code: 15030100R. ISSN: 0003-4975. Pub. country: United States. Language:

English.

AB BACKGROUND: Nafamostat mesilate (FUT-175) is a synthetic **serine protease inhibitor** that inactivates coagulation, fibrinolysis, and platelet **aggregation.** Nafamostat mesilate may suppress the blood-foreign surface reaction similar to biocompatible materials by blocking factor XIIa. **METHODS**: We performed an in vitro study of cardiopulmonary bypass (CPB) with fresh human blood among the following three groups: standard CPB sets (C), biocompatible CPB sets (B), and standard CPB sets with FUT-175 (10 mg/L) (F). A clinical study using these same CPB groups also was performed in 45 patients undergoing aortocoronary bypass operations (15 patients each). We injected FUT-175 at 40 mg/h during CPB. RESULTS: In the in vitro study, both groups B and F showed significantly lower levels of coagulation factors, thrombin-antithrombin III complex, fibrinopeptide A, beta-thromboglobulin, complement C3a, granulocyte elastase, and free hemoglobin than group C at the conclusion of the study. Thrombin-antithrombin III complex and free hemoglobin in group F also were lower than in group B. The platelet count remained at a higher level in group F than in the other groups. Separation of bradykinin was suppressed most significantly in group F. In the clinical study, group F also showed significantly lower levels of alpha 2-plasmin **inhibitor** plasmin complex and C3a than both groups C and B. There were minimal levels of free hemoglobin in group F. CONCLUSIONS: Nafamostat mesilate may contribute major beneficial effects toward conservation of blood during CPB and prevention of coagulopathy after CPB.

L24 ANSWER 21 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN

1995:761808 Document No. 123:164691 Blood coagulation retardants and devices. Lyon, Martha E.; Henderson, Paul; Malik, Sohail; Kenny, Margaret A.; Lyon, Andrew W. (University of Washington, USA). PCT Int. Appl. WO 9514788 A1 19950601, 27 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US13537 19941123. PRIORITY: US 1993-157880 19931124.

AB The invention provides **methods** of using anticoagulants to retard the coagulation of blood, so that properties and functions of blood, plasma, and blood cells may be determined anal. The **methods** do not interfere with electrochem. techniques use to detect divalent cations and permit accurate anal. of many analytes within a single blood sample, which currently require sep. anticoagulated blood samples. The **serine protease inhibitors** used may be combined with each other or blood cell activation, **aggregation**, and adhesion **inhibitors** in mixts. that provide anticoagulant activity. The **methods** permit, for the first time, the possibility of using a single blood sample to perform a full range of blood, plasma, and blood cell analyses. The anticoagulation effect of D-phenylalanyl-prolyl-arginyl chloromethyl ketone is determined

L24 ANSWER 22 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN

1995:721060 Document No. 123:144638 Preparation of peptide boronic acid derivatives having protease **inhibiting** activity. Cook, Nigel Scott; Metternich, Rainer; Tapparelli, Carlo; Wienand, Anette (Sandoz-Erfindungen Verwaltungsgesellschaft m.b.H., Austria; Sandoz-Patent-G.m.b.H.; Sandoz Ltd.). PCT Int. Appl. WO 9420526 A1 19940915, 42 pp. DESIGNATED STATES: W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-EP595 19940301. PRIORITY: GB 1993-4289 19930303; GB 1993-6822 19930401.

AB Peptidomimetics of formula W-Y-NR4CHR5BQ1Q2 [I; W = H, N-protective group; Y = a sequence of n amino acids such that the (n+1) amino acid peptide

Y-Lys or Y-Arg has an affinity for the active site of a trypsin-like protease, where n = an integer of 1-10 and in which at least one amino acid is an unnatural amino acid having a hydrophobic side chain; Q1, Q2 = OH, COR1, CONR1R2, NR1R2, or OR3 or Q1 and Q2 taken together form a diol residue; R1 - R3 = C1-10 alkyl, C6-10 aryl, C6-10 aralkyl, Ph substituted by up to 3 groups selected from C1-4 alkyl, halo, and C1-4 alkoxy; R4 = halo, C1-10 alkyl; R5 = A-X; A = (CH2)z (wherein z = 2-5), CHMeCH2CH2, CH2CHMeCH2CH2, CH2CH2CHMe, CH2CH2CMe2, CHMe(CH2)3, etc.; X = OH, SH, NR6R7; R6 = H, C1-10 alkyl; R7 = C1-10 alkyl, COR8, C(S)R8, SO2R8; R8 = H, C1-10 alkyl, C1-10 alkoxy, C6-10 aryl, (un)substituted C6-10 aralkyl], which are potent thrombin **inhibitors**, are prepared A therapeutic composition for **inhibition** of trypsin-like serine proteases such as thrombin, factor Xa, kallikrein, plasma prolyl endopeptidase, and IgA1 protease or having anticoagulant or anti-thrombogenic activity contains any one of peptide mimics I. These compds. I are used as thrombin **inhibitors** or **inhibitors** of vascular remodeling (proliferation, migration) following venous or arterial surgery or other forms of vascular damage. Thus, Boc-TMSal-Pro-NHCH[(CH2)4OH]BOPin (Boc = Me3CO2C, TMSal = trimethylsilylalanine, OPin = pinanediol) was prepared by the solution **method**, which in vitro showed the **inhibition** of α-thrombin with Ki of 13 nM and in the arterio-venous shunt thrombosis model in rats, **inhibited** thrombus formation by 21% at a dose of 0.3 mg/kg i.v. and blocked it at a dose of 3 mg/kg i.v.

L24 ANSWER 23 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1994:477585 Document No.: PREV199497490585. Purification and characterization of active and stable recombinant plasminogen-activator **inhibitor** accumulated at high levels in Escherichia coli. Sancho, Elena; Tonge, David W.; Hockney, Robert C.; Booth, Nuala A. [Reprint author]. Dep. Molecular Cell Biol., Univ. Aberdeen, Marischal Coll., Aberdeen AB9 1AS, UK. European Journal of Biochemistry, (1994) Vol. 224, No. 1, pp. 125-134.
CODEN: EJBCAI. ISSN: 0014-2956. Language: English.

AB Plasminogen-activator **inhibitor** type 1 (PAI-1), the primary physiological **inhibitor** of tissue-type plasminogen activator, is an unusual member of the **serine protease inhibitor** (serpin) superfamily in that it spontaneously converts to a latent form lacking activity. This latent form can be reactivated by denaturation and refolding, but the activation is usually incomplete and often leads to **aggregation** of the protein. In this study we have developed a high-level expression system that leads to the accumulation of PAI-1 at 30-50% total microbial protein. We have developed a single-step purification protocol which can be completed in a few hours, yielding approximately 20 mg purified recombinant PAI-1/litre culture. The purified PAI-1 was 80-100% active and was stable upon incubation at 37 degree C with a half-life of approximately 48 h. At 20 degree C, PAI-1 activity was stable for a week and at 4 degree C it retained its activity completely for up to two months. Freezing caused significant loss of activity. The stability of PAI-1 activity was found to be dependent on pH and ionic strength, being most stable at pH 5.6 and at an ionic strength of 1 M salt. We show that by a combination of high level expression and rapid purification under optimum conditions, it is possible to produce active and stable PAI-1 in high yield.

L24 ANSWER 24 OF 27 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1994:377395 Document No.: PREV199497390395. A randomised double blind study comparing KPI (a **serine protease inhibitor**) with aprotinin in reducing perioperative blood loss in an ovine model of cardiopulmonary bypass. Hunt, Beverley J. [Reprint author]; Parratt, Rachel [Reprint author]; Ohri, S.; Becket, J.; Brannan, J.; Taylor, K. M.. Res. Haematol., Harefield Hosp., Middlesex UB9 6JH, UK. British Journal of Haematology, (1994) Vol. 86, No. SUPPL. 1, pp. 65.
Meeting Info.: Annual Scientific Meeting of the British Society for Haematology. Harrogate, England, UK. April 25-28, 1994.
CODEN: BJHEAL. ISSN: 0007-1048. Language: English.

L24 ANSWER 25 OF 27 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4

93175928 EMBASE Document No.: 1993175928. α1-Antichymotrypsin binding to Alzheimer Aβ peptides is sequence specific and induces fibril disaggregation in vitro. Fraser P.E.; Nguyen J.T.; McLachlan D.R.; Abraham C.R.; Kirschner D.A.. Ctr. for Res. Neurodegenerative Dis., Tanz Neuroscience Building, University of Toronto, 6 Queen's Park Crescent West,Toronto, Ont. M5S 1A8, Canada. Journal of Neurochemistry 61/1 (298-305) 1993.
ISSN: 0022-3042. CODEN: JONRA. Pub. Country: United States. Language: English. Summary Language: English.

AB The **serine protease inhibitor** α1-antichymotrypsin (ACT) consistently colocalizes with amyloid deposits of Alzheimer's disease (AD) and may contribute to the generation of amyloid proteins and/or physically affect fibril assembly. AD amyloid fibrils are composed primarily of Aβ, which is a proteolytic fragment of the larger β-amyloid precursor protein. Using negative-stain and immunochemical electron microscopy, we have investigated the binding of ACT to the fibrils formed by four synthetic Aβ analogues corresponding to the wild-type human 1-40 sequence [H(wt)(1-40)], a 1-40 peptide [H(Du)(1-40)] containing the Glu22 → Gln mutation found in hereditary cerebral hemorrhage with amyloidosis of the Dutch type, the N-terminal 1-28 residues [β(1-28)], and an internal fragment of Aβ containing residues 11-28 [β(11-28)]. Each of these peptide analogues assembled into 70-90-Å-diameter fibrils resembling native amyloid and, except for β(11- 28), bound ACT, as indicated by the appearance of 80-100-Å globular particles that adhered to preformed fibrils and that could be decorated with anti-ACT antibodies. Under the conditions used, ACT binding destabilized the in vitro fibrils and produced a gradual dissolution of the macromolecular assemblies into constituent filaments and shorter fragments. The internal fragment (11-28) did not exhibit ACT binding or any structural changes. These results suggest that a specific sequence likely contained within the N- terminal 10 residues of Aβ is responsible for the formation of the ACT- amyloid complex. Although the observed fibril disassembly is surprising in view of the notion that ACT contributes directly to the physical process involved in amyloid fibril **formation, the** induced structural changes may expose new domains in Aβ for additional proteolysis or for interactions with cell-surface receptors.

L24 ANSWER 26 OF 27 MEDLINE on STN DUPLICATE 5

83285810. PubMed ID: 6349855. Molecular markers of hemostatic disorders: implications in the diagnosis and therapeutic management of thrombotic and bleeding disorders. Fareed J; Bick R L; Squillaci G; Walenga J M; Bermes E W Jr. Clinical chemistry, (1983 Sep) 29 (9) 1641-58. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB With current technological advances, it is now possible to measure in less than 50 microL of plasma picomolar amounts of circulating products of platelet activation, products of protease activation related to coagulation and fibrinolytic pathways, and prostaglandin metabolites formed during a physiologic or pathologic process. Most of these markers, which circulate in blood in nanogram or picogram amounts per milliliter during or after pathologic activation, provide pertinent information on the status of a patient in terms of specificity and early detection, and will be of crucial value in the diagnosis of hemostatic defects and the management of newer antithrombotic drugs that cannot be monitored by currently available assays. Currently, 125I- and 3H-based simple radioimmunoassays are available for platelet factor 4, beta-thromboglobulin, fibrinopeptide A, B beta 15-42 related peptides, thromboxane B2, and the prostaglandins 6-keto-PGF1 alpha and PGE2. Nonisotopic **methods** such as enzyme-linked immunosorbent assays and fluoroimmunoassays are being developed. Serotonin and ADP, products of platelet activation, are measurable by liquid-chromatographic, immunoenzymatic, and spectrophotofluorometric **methods.** Analytical **methods** for fibrin split products (fragments D and E)

and **serine protease inhibitor** complexes such as thrombin-antithrombin-III, factor Xa-antithrombin-III, and kallikrein-C1-esterase are also being developed. We have evaluated all of these **methods** and found them to be very sensitive to those components of endogenous activation of the hemostatic system listed above.

L24 ANSWER 27 OF 27 CAPLUS COPYRIGHT 2004 ACS on STN
1983:149488 Document No. 98:149488 In vitro studies of anti-**inhibitor** correctional concentrates with factor VIII bypass activity. Messmore, H. L., Jr.; Fareed, J.; Kelly, J.; Walenga, J.; Mazzucconi, M. G. (Med. Cent., Layola Univ., Maywood, IL, USA). Act. Prothrombin Complex Conc., [Proc. Lect. Pap. Meet.], Meeting Date 1981, 88-94. Editor(s): Mariani, Guglielmo; Russo, M. A.; Mandelli, Franco. Praeger: New York, N. Y. (English) 1982. CODEN: 49IGA5.
AB Active principles in com. activated prothrombin complex concns. (APCC), Autoplex and antiinhibitor correctional concentrate (AICC), were evaluated using clotting and amidolytic **methods.** The concs. were tested for their protein content, coagulation factors II [9001-26-7], VII [9001-25-6], IX [9001-28-9] and X [9001-29-0] and **serine protease inhibitors** antithrombin [9000-94-6] III, α2-antiplasmin, α2-macroglobulin, α1-antitrypsin [9041-92-3] and C1'-1 esterase **inhibitor.** All the concs. contained some thrombin like activity. The greatest thrombin-like activity was seen in AICC which contains no heparin. The release and **aggregation** of blood platelets was blocked with heparin. The coagulation factors in prothrombin complex concs. were compared with those in the APCC and the differences were in the high levels of factors XII and IX in nonactivated concs. Thus, the active principle in the factor VIII [9001-27-8] bypassing concs. was not clearly identified.

=> s l3 and crystalline
L25 168 L3 AND CRYSTALLINE

=> s l25 and inhibitor
L26 21 L25 AND INHIBITOR

=> dup remove l26
PROCESSING COMPLETED FOR L26
L27 16 DUP REMOVE L26 (5 DUPLICATES REMOVED)

=> d l27 1-16 cbib abs

L27 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
2003:950061 Document No. 140:8764 Polymorphs of clopidogrel hydrogen sulfate. Lifshitz-Liron, Revital; Kovalevski-Ishai, Eti; Wizel, Shlomit; Avhar-Maydan, Sharon; Lidor-Hadas, Rami (Israel). U.S. Pat. Appl. Publ. US 2003225129 A1 20031204, 31 pp., Cont.-in-part of U.S. Ser. No. 74,409. (English). CODEN: USXXCO. APPLICATION: US 2003-339008 20030108. PRIORITY: US 2002-PV348182 20020111; US 2002-74409 20020212; US 2002-PV359157 20020221; WO 2002-US40679 20021218.
AB Provided are new **crystalline** Forms III, IV, V and VI of clopidogrel hydrogen sulfate and the amorphous form of clopidogrel hydrogen sulfate, as well as their pharmaceutical compns., and **method** of treatments with such compns. Also provided are novel processes for the preparation of clopidogrel hydrogen sulfate Form I, Form II, Form III, Form IV, Form V, Form VI and amorphous form. Clopidogrel base (4.27 g) was dissolved in Me Et ketone (MEK) (33.7 mL). Eighty percent aqueous H_2SO_4 (1.03 mL) was added to the solution at 20°. The reaction mixture was heated to reflux temperature for 2 h and then the solution was cooled to room temperature and stirred at this temperature for addnl. 67 h during which a precipitate was formed. The white solid was collected by filtration, washed with MEK and dried at

50° in a vacuum oven for 24 h to obtain 4.59 g (82%) of clopidogrel hydrogen sulfate crystal Form II.

L27 ANSWER 2 OF 16 MEDLINE on STN
2003433141. PubMed ID: 12974387. The molecular chaperone, alpha-crystallin, protects against loss of antigenicity and activity of esterase caused by sugars, sugar phosphate and a steroid. Yan Hong; Harding John J. (Nuffield Laboratory of Ophthalmology, University of Oxford, Walton St., Oxford OX2 6AW, UK.) Biological chemistry, (2003 Aug) 384 (8) 1185-94. Journal code: 9700112. ISSN: 1431-6730. Pub. country: Germany: Germany, Federal Republic of. Language: English.
AB Previously we showed that glycation-induced inactivation and loss of antigenicity of enzymes occur simultaneously. Alpha-crystallin, a major structural protein of the mammalian lens, prevents the **aggregation** of other proteins and protects enzyme function against post-translational modification in vitro. However, it is not known whether alpha-crystallin can also protect against loss of antigenicity of enzymes. Esterase activity in the lens is decreased in senile cataract and diabetes. We investigated the loss of antigenicity of esterase caused by different insults and the ability of alpha-crystallin to protect. Inactivation of carboxylesterase by sugars, fructose 6-phosphate (F6P) and a steroid, prednisolone-21-hemisuccinate (P-21-H), was measured spectrophotometrically in the presence and absence of alpha-crystallin, while loss of antigenicity was monitored simultaneously using an immunoprecipitation **method.** The esterase was progressively inactivated by fructose, F6P, ribose, and P-21-H. Bovine alpha-crystallin fully protected against inactivation of esterase by all four compounds, and also protected against loss of antigenicity of the esterase by fructose, ribose and P-21-H at a molar ratio of 1:1. The results indicated that alpha-crystallin, under our experimental conditions, clearly exhibited the ability to prevent loss of antigenicity and inactivation of esterase. The protective effect of alpha-crystallin against loss of antigenicity indicates a novel aspect of its chaperoning function.

L27 ANSWER 3 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:500024 Document No.: PREV200200500024. Characterization and crystal structure of a high-affinity pentavalent receptor-binding **inhibitor** for cholera toxin and E. coli heat-labile enterotoxin. Merritt, Ethan A.; Zhang, Zhongsheng; Pickens, Jason C.; Ahn, Misol; Hol, Wim G. J. [Reprint author]; Fan, Erkang. Department of Biochemistry, Biomolecular Structure Center, University of Washington, Box 357742, Seattle, WA, 98195, USA. hol@gouda.bmsc.washington.edu. Journal of the American Chemical Society, (July 31, 2002) Vol. 124, No. 30, pp. 8818-8824. print.
CODEN: JACSAT. ISSN: 0002-7863. Language: English.
AB Multivalent ligand design constitutes an attractive avenue to the **inhibition** of receptor recognition and other biological events mediated by oligomeric proteins with multiple binding sites. One example is the design of multivalent receptor blockers targeting members of the AB5 bacterial toxin family. We report here the synthesis and characterization of a pentavalent **inhibitor** for cholera toxin and Escherichia coli heat-labile enterotoxin. This **inhibitor** is an advance over the symmetric pentacyclen-derived **inhibitor** described in our earlier work in that it presents five copies of m-nitrophenyl-alpha-D-galactoside (MNPG) rather than five copies of beta-D-galactose. The approximately 100-fold higher single-site affinity of MNPG for the toxin receptor binding site relative to galactose is found to yield a proportionate increase in the affinity and IC50 measured for the respective pentavalent constructs. We show by dynamic light scattering that **inhibition** of receptor binding by the pentavalent **inhibitor** is due to 1:1 **inhibitor**:toxin association rather than to **inhibitor**-mediated **aggregation.** This 1:1 association is in complete agreement with a 1.46 ANG resolution crystal structure of the pentavalent **inhibitor**

:toxin complex, which shows that the favorable single-site binding interactions of MNPG are retained by the five arms of the 5256 Da pentavalent MNPG-based **inhibitor** and that the initial segment of the linking groups interacts with the surface of the toxin B pentamer.

L27 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
2001:283768 Document No. 134:300799 Bioadhesive nanoparticulate compositions having cationic surface stabilizers. Bosch, H. William; Cooper, Eugene R. (Elan Pharma International Ltd., Ire.; McGurk, Simon L.). PCT Int. Appl. WO 2001026635 A2 20010419, 93 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US27434 20001005. PRIORITY: US 1999-414159 19991008.

AB Bioadhesive nanoparticulate compns., comprising active agent particles and one or more cationic surface stabilizers, are described. The cationic surface stabilizers prevent **aggregation** of the nanoparticles and increase bioadhesion of the nanoparticles to biol. substrates, such as an insect, teeth, bone, nails, chitin, feathers, scales, mucous, skin, hair, plant tissue, etc. The particles may consist of pharmacol. active compds. (e.g., drugs for human or veterinary use), agricultural chems. (pesticides, herbicides, fertilizers, and the like), cosmetics, consumer products (coloring agents, flavors, or fragrances), or other materials which function by interacting with biol. substrates. In addition, the invention relates to **methods** of preparing and using such bioadhesive nanoparticulate compns. Thus, a first nanoparticulate formulation was prepared having a ratio of 30:3 naproxen to PVP (a nonionic surface stabilizer) and a second nanoparticulate formulation was prepared having a ratio of 10:1 naproxen and polymethyl methacrylate trimethylammoniumbromide bromide as a surface stabilizer. This nanoparticulate composition utilizing a cationic surface stabilizer and a **crystalline** agent exhibits increased bioadhesion to plant tissue as compared to conventional nanoparticulate compns. comprising a noncationic surface stabilizer.

L27 ANSWER 5 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:217083 Document No.: PREV200100217083. Calpain-induced light scattering in young rat lenses is enhanced by UV-B. Nakamura, Yoshikuni; Fukiage, Chiho; Azuma, Mitsuyoshi [Reprint author]; Shearer, Thomas R.. Research Laboratory, Senju Pharmaceutical Corp., Ltd., Kobe, 651-2241, Japan. mitsuyoshi-azuma@senju.co.jp. Journal of Ocular Pharmacology and Therapeutics, (February, 2001) Vol. 17, No. 1, pp. 47-58. print. ISSN: 1080-7683. Language: English.

AB The purpose of this study is to determine if UV-B enhances light scattering after proteolysis of crystallins by calpains, and to determine if lens-specific calpain Lp82 is involved, along with m-calpain, in the mechanism of in vitro precipitation. Lens soluble proteins from young rats were hydrolyzed for 24 hr by endogenous lens calpains, and the proteins were further incubated for up to 7 days with periodic irradiation by UV-B. Light scattering was measured daily at 405 nm. SDS-PAGE and immunoblotting assessed proteolysis of crystallins, activation of calpains, and formation of high molecular weight **aggregations.** Appreciable light scattering occurred in lens soluble proteins after proteolysis of crystallins by m-calpain and Lp82. UV-B markedly enhanced this light scattering and the formation of higher molecular weight aggregates consisting of proteolyzed alpha- and beta- and intact gamma-crystallins. Calpain **inhibitor** E64 and antioxidants DTE or GSH prevented the light scattering. These results show that calpain-induced light scattering is enhanced by the natural oxidant UV-B. Activation of Lp82, along with m-calpain, contributed to the light

scattering. The linkage between proteolysis and oxidation is important because both oxidation and truncation of crystallins are found in aged human lenses, which are constantly exposed to UV irradiation.

L27 ANSWER 6 OF 16 MEDLINE on STN
2001252967. PubMed ID: 11262616. Comparison of various calpain **inhibitors** in reduction of light scattering, protein precipitation and nuclear cataract in vitro. Mathur P; Gupta S K; Wegener A R; Breipohl W; Ahrend M H; Sharma Y D; Gupta Y K; Vajpayee R B. (Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-29, India.) Current eye research, (2000 Dec) 21 (6) 926-33. Journal code: 8104312. ISSN: 0271-3683. Pub. country: England: United Kingdom. Language: English.

AB PURPOSE: To compare effects of calpain **inhibitors** on in vitro light-scattering in rat lens soluble protein and calcium-ionophore (A23187)-induced cataract formation in cultured rat lenses. **METHODS**: Rat lens soluble protein was hydrolyzed for 24 hours by activation of endogenous lens calpain. Ten calpain **inhibitors** were tested in this model at 10 and 25 microM concentration. As an index of protein precipitation, light scattering was measured daily at 405 nm for 8 days. Lens proteins were analyzed by isoelectric-focussing. Subsequently, rat lenses were cultured for 5 days with 10 microM A23187. Calpain **inhibitors** (SJA6017, MDL28170, AK295 and PD150606), which **inhibited** light-scattering were tested at 100 microM concentration in this model. Cataract evaluation, isoelectric-focussing and calcium determinations were performed. RESULTS: At 25 microM concentration AK295, SJA6017, E-64, PD-150606 and MDL28170 produced greater than 25% **inhibition** of light-scattering. Isoelectric-focussing revealed that addition of Ca(2+) produced characteristic crystallin proteolysis and **aggregation** patterns. AK295, SJA6017, MDL28170 and E64c prevented these changes. Lenses cultured in A23187 exhibited nuclear cataract, elevated calcium and proteolysis and **aggregation** of crystallins. Co-culture with SJA6017, MDL28170 and E64c reduced A23187-induced nuclear opacities, proteolysis and **aggregation** of crystallins without affecting increased total calcium. CONCLUSIONS: Endogenous calpain-activation model and A23187-induced cataract model can be used sequentially to screen calpain **inhibitors** for potential anti-cataract activity. Proteolytic changes in lens cortex after exposure to A23187 are also due to calpain activation. AK295, SJA6017 and MDL28170 possess efficacy against calcium-induced models of rodent cataracts. Use of calpain **inhibitors** represents a promising approach to cataract therapy.

L27 ANSWER 7 OF 16 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 1
2001:83036 The Genuine Article (R) Number: 391DD. Studying noncovalent protein complexes in aqueous solution with laser desorption mass spectrometry. Wattenberg A; Sobott F; Barth H D; Brutschy B (Reprint). Univ Frankfurt, Inst Theoret & Phys Chem, Marie Curie Str 11, D-60439 Frankfurt, Germany (Reprint); Univ Frankfurt, Inst Theoret & Phys Chem, D-60439 Frankfurt, Germany. INTERNATIONAL JOURNAL OF MASS SPECTROMETRY (29 DEC 2000) Vol. 203, No. 1-3, pp. 49-57. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 1387-3806. Pub. country: Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The study of noncovalent **aggregation** with mass spectrometry has been largely the domain of electrospray ionization mass spectrometry (ESI-MS). In contrast, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has been applied to this field to a much lesser extent. The main drawback of MALDI-MS is that the sample preparation requires a **crystalline** matrix. This disrupts the solution environment and often leads to dissociation of noncovalent complexes. A new laser desorption **method**, developed in our group, promises to circumvent this shortcoming. It is called laser induced liquid beam ionization/desorption mass spectrometry (LILBID-MS). The major advantage of this new **method** is the use of a liquid beam in vacuum for

sample preparation and as target. The beam is directly injected into the mass spectrometer, using the solvent (mostly water) as the natural matrix substance, thus allowing for a softer probe preparation and desorption. In this article we present examples for the application of this new desorption **method** for detecting noncovalent aggregates of proteins in aqueous solutions. Using ribonuclease S, calmodulin/melittin, and bovine pancreatic trypsin **inhibitor** as model systems, evidence is given that LILBID-MS is capable of desorbing intact noncovalent complexes into the gas phase. Even water bound into cavities of a protein structure can be detected. In addition, it will be shown that solution parameters (e.g. pH, temperature) have a decisive influence on the mass spectra obtained, thus confirming earlier observations that the ions detected by LILBID-MS are formed in the solution phase and are not gas phase artifacts produced by the detection process. (C) 2000 Elsevier Science B.V.

L27 ANSWER 8 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:151333 Document No.: PREV199900151333. Lesions and identification of **crystalline** precipitates of glycoprotein IIb-IIIa antagonists in the rat kidney. Levin, Stuart; Friedman, Robert M.; Cortez, Enriqueta; Hribar, Jeremy; Nicholas, Mark; Schlessinger, Sally; Fouant, Monique; Khan, Nasir. Monsanto/Searle Research Development, 4901 Searle Parkway, Skokie, IL 60077, USA. Toxicologic Pathology, (Jan.-Feb., 1999) Vol. 27, No. 1, pp. 38-43. print.
CODEN: TOPADD. ISSN: 0192-6233. Language: English.

AB Two glycoprotein IIb-IIIa antagonists (xemilofiban, SC-54684A, and orbofiban, SC-57099B), which are platelet **aggregation inhibitors**, caused **crystalline** precipitates in the kidney tubules of rats at high dosages. Dogs were not affected. Depending on the degree of the precipitation, which was dosage dependent, and the location, which differed somewhat between the two compounds, the lesions varied from acute obstruction with tubule cell necrosis, nephron dilation, and sudden death with no inflammation to severe chronic pyogranulomatous inflammation. In order to understand the relevance of the lesions, it was important to identify the precipitates. This was technically challenging because the crystals were water soluble (dissolving in routine fixing and staining techniques) and were present in insufficient quantity to physically isolate. Techniques were devised to evaluate the crystals in situ in unstained frozen sections prepared without directly embedding the tissues in supporting medium, which interfered with the analyses. The crystals were analyzed in situ by infrared and Raman spectroscopy and time-of-flight secondary ion mass spectroscopy (TOF-SIMS). Uroliths found in the renal pelvis of one animal were analyzed by liquid chromatography/mass spectrometry. The resulting spectra showed that the crystals were the de-esterified acids of the parent compounds. This knowledge allowed us to predict that the **crystalline** precipitates would not be a hazard to humans because of the large multiples of the human dosage at which they occurred and because of differences in renal physiology between rats, dogs, and humans.

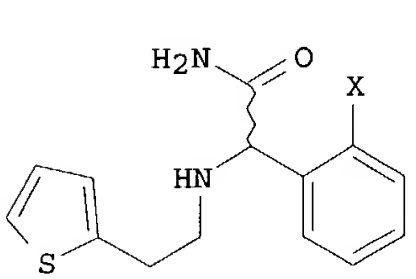
L27 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
1999:9822 Document No. 130:71532 **Crystalline** roxifiban for the treatment of platelet **aggregation**-mediated diseases. Maurin, Michael B.; Ma, Philip; Meloni, David J.; Pesti, Jaan A.; Rossano, Lucius T.; Ward, Randall K.; Yin, Jianguo; Zhang, Lin-hua (The Du Pont Merck Pharmaceutical Company, USA). PCT Int. Appl. WO 9857939 A1 19981223, 48 pp. DESIGNATED STATES: W: AU, BR, CA, CN, CZ, EE, HU, IL, JP, KR, LT, LV, MX, NO, NZ, PL, RO, SG, SI, SK, UA, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US12367 19980612. PRIORITY: US 1997-49712 19970616; US 1997-49633 19970616; US 1998-80278 19980401.

AB The potent platelet glycoprotein IIb/IIIa antagonist, roxifiban, is prepared in **crystalline** forms. **Crystalline** roxifiban exists in two polymorphic forms, designated Form 1 and Form 2. These polymorphic forms

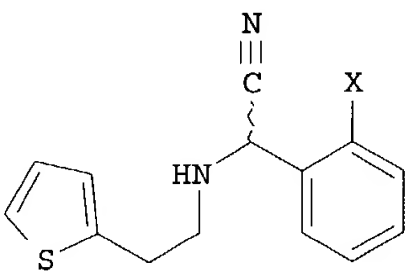
are characterized by x-ray powder diffraction and solid-state carbon NMR. Pharmaceutical compns. and **methods** for the treatment or prevention of diseases mediated by platelet **aggregation** are described.

L27 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
1998:761891 Document No. 130:24963 New 2-[(2-thienyl)ethylamino](2-halophenyl)acetamide intermediates for clopidogrel and analogs, and process for their preparation. Bakonyi, Maria; Csatari Nagy, Marianna; Molnar, Levente, Mrs.; Makovi, Zoltan; Jobb, Piroska; Bai, Tibor, Mrs. (Sanofi, Fr.). PCT Int. Appl. WO 9851681 A1 19981119, 24 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-HU47 19980511. PRIORITY: HU 1997-9700884 19970513.

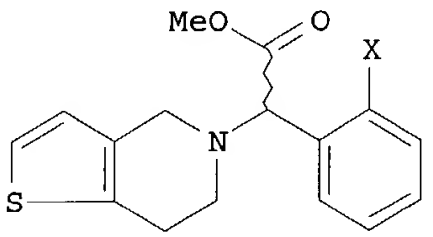
GI



I



II



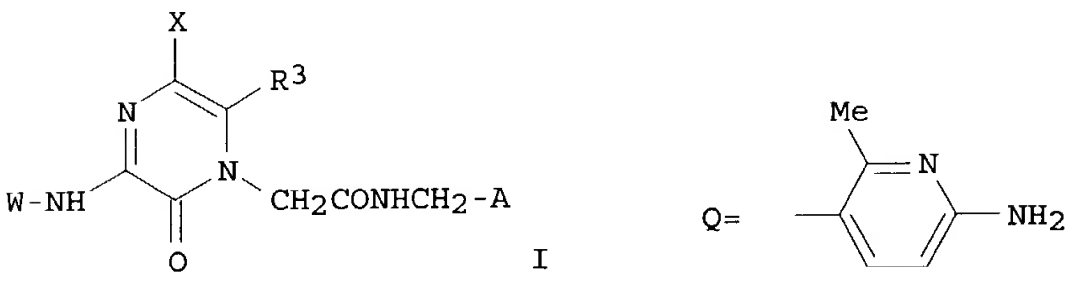
III

AB A process for the preparation of [[2-(2-thienyl)ethyl]amino](2-halophenyl)acetamides I [X = halo] from nitriles II is disclosed. I are valuable intermediates for Me (2-halophenyl)(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetates III and their salts, e.g., the drug clopidogrel, which have platelet-**aggregation-inhibitory** and antithrombotic activity (no data). The **method** eliminates the use of lacrimatory and irritant α-halophenylacetic acid derivs. as intermediates. For instance, II [X = Cl] in MeOAc at 15-25° was treated with HCl gas and then MeOH to give after 6 h a precipitate of **crystalline** I.HCl [X = Cl] in 94% yield. This amide was hydrolyzed with H2SO4 in MeOH to give the corresponding Me ester hydrochloride (82%), which was cyclized with paraformaldehyde in formic acid to give the HCl salt of clopidogrel racemate, i.e., III.HCl [X = Cl], in 86.6% yield. A total of 22 examples illustrate both racemic and optically active variations of different steps in the overall process.

L27 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
1997:717898 Document No. 128:22922 Preparation of 3-amino-2-pyrazinone-1-acetamide derivatives as thrombin **inhibitors.** Sanderson, Philip E.; Lyle, Terry A.; Dorsey, Bruce D.; Varsolona, Richard J. (Merck & Co., Inc., USA; Sanderson, Philip E.; Lyle, Terry A.; Dorsey, Bruce D.;

Varsolona, Richard J.). PCT Int. Appl. WO 9740024 A1 19971030, 193 pp. DESIGNATED STATES: W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US6744 19970418. PRIORITY: US 1996-16041 19960423; GB 1996-9714 19960509; US 1997-43009 19970414.

GI



I

AB Compds. of general formula [I; W = H, R1, R1O2C, R1CO, R1(CH2)nNHCO, (R1)2CH(CH2)nNHCO; wherein n = 1-4; R1 = R2, R2 (CH2)m C(R12)2, R2CH(OR2)(CH2)p, R2C(R12)2(CH2)m, R2CH2C(R12)2(CH2)q, (R2)2CH(CH2)r, R2O(CH2)p, R2(CO2R3)(CH2)s, etc.; wherein p, s = 1-4; m = 0-3; q = 0-2; r = 0-4; R2 = (un)substituted Ph, naphthyl, biphenyl, (un)substituted and (un)saturated 5- to 7-membered mono- or 9- to 10-membered bicyclic heterocyclic ring or non-heterocyclic ring, wherein the heterocyclic ring contains 1-4 heteroatoms selected from N, O, and S; R3 = H, C1-4 alkyl, C3-7 cycloalkyl, CF3; X = H, halo; ring-(un)substituted 2-amino-5-pyridyl or 2-amino-4-pyridyl, (un)substituted Ph] are prepared These compds. are useful in **inhibiting** thrombin (serine protease) and associated thrombotic occlusions. This invention also includes a pharmaceutical composition containing I for **inhibiting** thrombus formation and a **method** for **inhibiting** thrombin in blood and formation of blood platelet aggregates by adding the composition to the blood and also a **method** for **inhibiting** thrombus formation by adding the composition to the blood and/or with a fibrinogen receptor antagonist. A **method** for treating or preventing venous thromboembolism and pulmonary embolism, deep vein thrombosis, cardiogenic thromboembolism, thromboembolic stroke, thrombus associated with cancer and cancer chemotherapy, unstable angina, myocardial infarction, cardiogenic thromboembolism associated with atrial fibrillation, prosthetic heart valves, or heart disease, atherosclerosis, etc. in a mammal by administering the composition is claimed. Thus, 3-(2-phenethylamino)-6-methyl-1-carboxypyridine was condensed with 2-amino-5-aminomethyl-6-methylpyridine dihydrochloride using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT.H2O, and N-methylmorpholine in DMF for 16 h to give I (W = CH2CH2Ph, X = H, R3 = Me, A = Q) (II). II in vitro **inhibited** human α-thrombin with Ki of ≤1 nM. A polymorphic **crystalline** form type A and type B monohydrate of II.2HCl were also prepared and claimed. Pharmaceutical compns., e.g. an tablet formulation containing II, were described.

L27 ANSWER 12 OF 16 MEDLINE on STN DUPLICATE 2

96387992. PubMed ID: 8795393. The effect of heparan sulphate on the crystallization of calcium oxalate in undiluted, ultrafiltered human urine. Suzuki K; Ryall R L. (Department of Surgery, Flinders Medical Centre, Bedford Park, South Australia, Australia.) British journal of urology, (1996 Jul) 78 (1) 15-21. Journal code: 15740090R. ISSN: 0007-1331. Pub. country: ENGLAND: United Kingdom. Language: English.

AB OBJECTIVE: To determine the effect of physiological concentrations of heparan sulphate (HS) on the crystallization of CaOx in undiluted,

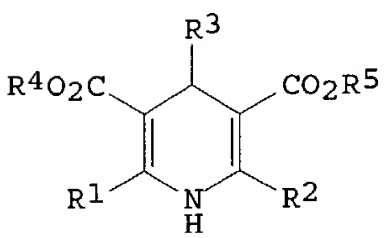
ultrafiltered human urine. SUBJECTS AND **METHODS**: Urine was collected from normal male volunteers and ultrafiltered at a nominal molecular weight threshold of 10 kDa. Calcium oxalate (CaOx) crystallization was induced by the addition of a standard load of oxalate above an experimentally determined metastable limit and the effect of added HS tested at concentrations of 0, 1, 2 and 10 micrograms/mL using a Coulter Counter particle analyser and scanning electron microscopy. RESULTS: The metastable limit of the urine for CaOx was unaffected by HS at any concentration, as was the total volume of **crystalline** material deposited. However, HS strongly **inhibited** the formation of crystal aggregates with a response dependent on the dose and completely prevented the formation of crystal aggregates at a final concentration of 10 micrograms/mL. These findings were confirmed by scanning electron microscopy. CONCLUSION: HS is a potent **inhibitor** of CaOx crystallization in human urine and may protect against CaOx stone formation by reducing the degree of **aggregation** and thereby, the size of particles precipitated in the urinary tract.

L27 ANSWER 13 OF 16 MEDLINE on STN DUPLICATE 3
93151863. PubMed ID: 1492839. Molecular structure and antiaggregating activity of the potent local anaesthetic (-)-4-[2-hydroxy-3-(N-isopropylamino)-propoxyimino]-cis-carane. Czarnecki R; Czerwinska K; Grochowska K; Grochowski J; Librowski T; Serda P. (Chair of Pharmacodynamics, Medical Academy, Krakow, Poland.) Arzneimittel-Forschung, (1992 Nov) 42 (11) 1279-83. Journal code: 0372660. ISSN: 0004-4172. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The molecular structure of (-)-4-[-2-hydroxy-3-(N-isopropylamino)-propoxyimino]-cis-carane ($C_{16}H_{30}O_2N_2.HCl$), a recently synthesized potent local anaesthetic, including the absolute configuration at 4 chirality centres was determined using X-ray diffraction **method.** The substance crystallizes in diastereoisomeric form in lowest symmetry (triclinic P1 space group). Determined intermolecular close contacts between chlorine atoms and nitrogen and hydroxyl oxygen are the main determinants of crystal packing. In the **crystalline** state nitrogen of the isopropylamine group has quaternary coordination. The influence of the title compound on blood platelets **aggregation** induced by adenosine diphosphate was studied. The results of parallel tests conducted for lidocaine and bupivacaine show that the antiaggregating activity of the title compound is much stronger. This property could be attributed to the monoterpene part of its molecule, in analogy to the observed cyclic adenosine monophosphate **inhibitory** action of forskolin (diterpene).

L27 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
1986:19517 Document No. 104:19517 Dihydropyridine derivatives and their salts. Tamada, Shigeharu; Nagami, Kazuyoshi; Teramoto, Shuji; Tanaka, Tatsuyoshi; Nakagawa, Kazuyuki (Otsuka Pharmaceutical Co., Ltd., Japan). Eur. Pat. Appl. EP 145434 A2 19850619, 136 pp. DESIGNATED STATES: R: CH, DE, FR, GB, IT, LI, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1984-308386 19841203. PRIORITY: JP 1983-228880 19831202.

GI



I

AB Dihydropyridines I (R1 and R4 = alkyl; R2 = alkyl or CH2XR6; X = unsatd.

hydrocarbondiyl which may have an O or NR7 interrupter; R6 = OH-(un)substituted Ph; R7 = alkyl; R3 = (un)substituted Ph; R5 = alkyl, (un)substituted tetrahydropyridinyl, etc.) and salts prepared by several **methods** are useful as hypotensives, vasodilators, antiinflammatories, phosphodiesterase **inhibitors**, peroxidized lipid metabolism lowering agents, and for prophylaxis and treatment of thrombosis. Thus, Me 3-[4-[(tetrahydropyranyl)oxy]phenyl]-2-(E)-propenyl 1,4-dihydro-3,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate, prepared by esterification of (E)-4-[(tetrahydropyran-2-yl)oxy]cinnamyl alc. with 1,4-dihydro-2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)pyridine-3-carboxylic acid, was hydrolyzed to Me 3-(4-hydroxyphenyl)-2-(E)-propenyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (I; R1 = R2 = R4 = Me, R3 = 4-O2NC6H4, R5 = CH2CH:CHC6H4OH-4) (II). II showed vasodilating activity in spontaneously hypertensive rats. A formulation for 1000 tablets contained II 5, lactose 50, cornstarch 25, **cryst** . cellulose 25, Me cellulose 1.5, and Mg stearate 1.0 g.

L27 ANSWER 15 OF 16 MEDLINE on STN

81172270. PubMed ID: 6783814. **Aggregation** of tobacco mosaic virus by sodium chondroitin sulfate. Sano Y; Inoue H. Microbiology and immunology, (1980) 24 (11) 1035-42. Journal code: 7703966. ISSN: 0385-5600. Pub. country: Japan. Language: English.

AB The precipitation of tobacco mosaic virus by sodium chondroitin sulfate in an aqueous solution was investigated kinetically by means of turbidimetry. The virus solution became turbid after the addition of chondroitin sulfate. A threshold concentration of chondroitin, 1.33 mg/ml, was required for virus precipitation, irrespective of the virus concentration. The precipitation resulted from a mutual spatial exclusion phenomenon, leading to the separation of the virus as a **crystalline** phase. The dimension of chondroitin sulfate calculated at the threshold concentration agreed well with that obtained by other **methods.** The initial slopes and the **aggregation** half-times of the virus aggregates depended on both chondroitin and virus concentrations and the former increased with the increase in concentration of each. Above the threshold concentration of chondroitin sulfate, tobacco mosaic virus **aggregation** was a rapid-**aggregation** process and ended within 100 sec.

L27 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

1972:95211 Document No. 76:95211 Influence of carbonylcyanide-m-chlorophenylhydrazone and 3-(3,4-dichlorophenyl)-1,1-dimethylurea on the fusion of primary thylakoids and the formation of **crystalline** fibrilsin bean leaves partially greened in far red light. Butler, W. L.; De Greef, J.; Roth, T. F.; Oelze-Karow, H. (Revelle Coll., Univ. California, La Jolla, CA, USA). Plant Physiology, 49(1), 102-4 (English) 1972. CODEN: PLPHAY. ISSN: 0032-0889.

AB Carbonylcyanide m-chlorophenylhydrazone (I) [555-60-2] at 10μM or 0.1mM prevented the white light-induced fusion of primary thylakoids formed in bean leaves partially greened in far red light. I also induced formation of **crystalline** rods in the leaves, apparently by causing precipitation of Fraction 1 protein. Another photophosphorylation **inhibitor**, 3-(3,4-dichlorophenyl)-1,1-dimethylurea [330-54-1], had no effect on thylakoids fusion induced by white light. Perhaps this compound does not inhibit high energy intermediates formed in cyclic photophosphorylation that are inhibited by I.

```
=> s l3 and amino acid substitution
L28          147 L3 AND AMINO ACID SUBSTITUTION

=> s l28 and peptide analog
L29            0 L28 AND PEPTIDE ANALOG

=> s l28 and screening inhibitor
L30            0 L28 AND SCREENING INHIBITOR
```

```
=> s l28 and inhibitor
L31          18 L28 AND INHIBITOR

=> dup remove l31
PROCESSING COMPLETED FOR L31
L32          15 DUP REMOVE L31 (3 DUPLICATES REMOVED)

=> d l32 1-15 cbib abs

L32  ANSWER 1 OF 15  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2004101678  EMBASE  Towards understanding the structure-function relationship
     of human amyloid disease. Dealwis C.; Wall J.. C. Dealwis, Dept. of
     Biochem. Cell./Molec. Biol., University of Tennessee, Knoxville, TN,
     United States. cdealwis@utk.edu. Current Drug Targets  5/2 (159-171)
     2004.
     Refs: 131.
     ISSN: 1389-4501. CODEN: CDTUAU. Pub. Country: Netherlands. Language:
     English. Summary Language: English.
AB   Immunoglobulin light chain (LC) proteins exhibit the greatest sequence
     variability of all proteins associated with amyloid disease. The hallmark
     event in amyloidogenesis is a change in the secondary and/or tertiary
     structure or a normal, soluble protein, that fosters self-aggregation and
     fibril formation. The structural heterogeneity of light
     chain proteins has hampered understanding of the precise mechanisms
     involved in fibril formation. The development of
     effective therapeutics will be benefited by a fundamental understanding of
     mechanisms and structural prerequisites which govern amyloidogenesis. This
     review focuses on light chain (AL) amyloidosis resulting from the
     aggregation of κ and λ LCs. Specifically the thermodynamic
     and structural data of several WT and mutant amyloidogenic LCs have been
     carefully examined. Moreover, we discuss the importance of hydrophobic and
     ionic interactions on amyloidosis by comparing several available
     three-dimensional structures of amyloidogenic and highly homologous
     non-amyloidogenic proteins that can be destabilized to become
     amyloidogenic by site specific mutations. .COPYRGT. 2004 Bentham Science
     Publishers Ltd.

L32  ANSWER 2 OF 15  CAPLUS  COPYRIGHT 2004 ACS on STN
2003:511501  Document No. 139:79148  Substitution and deletion variants of the
     transglutaminase-inhibiting tridegins for use as anticoagulants.
     Giersiefen, Helmut; Stoeckel, Johannes; Pamp, Tanja; Ohlmann, Marion
     (Curacyte A.-G., Germany).  PCT Int. Appl. WO 2003054194 A2 20030703, 54
     pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR,
     BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB,
     GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
     LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL,
     PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US,
     UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT,
     BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE,
     IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (German).  CODEN:
     PIXXD2.  APPLICATION: WO 2002-EP14684 20021220.  PRIORITY: DE
     2001-10163333 20011221; DE 2002-10258159 20021212.
AB   The invention relates to substitution derivs. of tridegin of Haementeria
     ghilianii in which a cysteine, either alone or combination with
     substitutions at any of positions lysine-2, lysine-7, histidine-10,
     glycine-12, leucine-24, tyrosine-31, phenylalanine-34, arginine-39,
     isoleucine-45, methionine-48, aspartic acid-50, proline-55,
     phenylalanine-58, asparagine-60, proline-65, arginine-66, and N- or
     C-terminal deletions that leave at least the amino acid sequence
     DDIYQRXVXFPXLPL intact are described for use in the therapeutic
     inhibition of factor XIIIa.  The derivs. may also be conjugated
     with polyethylene glycol.  The invention further relates to
     methods for production of said inhibitors and the use
```

thereof as transglutaminase **inhibitors.** Testing of deletion derivs. of the protein and manufacture of larger peptides in Komagataella pastoris is demonstrated. Deletion derivs. with up to 80% of the **inhibitory** activity of the full-length protein are identified.

L32 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2004:265859 Document No.: PREV200400266576. Mutations within the cyclooxygenase-1 gene in aspirin non-responders with recurrence of stroke. Hillarp, Andreas [Reprint Author]; Palmqvist, Barbro; Lethagen, Stefan; Villoutreix, Bruno O.; Mattiasson, Ingrid. Dept Clin Chem, Malmo Univ Hosp, SE-20502, Malmo, Sweden. andreas.hillarp@klkemi.mas.lu.se. Thrombosis Research, (2003) Vol. 112, No. 5-6, pp. 275-283. print. CODEN: THBRAA. ISSN: 0049-3848. Language: English.

AB Introduction: Aspirin is a common antiplatelet drug used in the prevention of ischemic stroke due to its **inhibitory** effect on platelet cyclooxygenase-1 (Cox-1). Patients can be categorized as either aspirin 'responders' or 'non-responders' depending on whether they are protected against a secondary stroke event or not. In this study, we have searched for variants of the Cox-1 gene that could possibly result in an unblocked and thus, aspirin-resistant Cox-1 enzyme and phenotype. Materials and **methods**: The Cox-1 gene was sequenced in 68 patients with recurrent ischemic stroke despite taking aspirin. The genotype distribution of identified variants was determined and compared with healthy control subjects. Mutations that involved **amino acid substitutions** of the mature Cox-1 molecule were analysed by molecular modelling and functional analysis using whole blood aggregometry. Results: Fourteen variants of the Cox-1 gene were identified. Seven of the variants involved **amino acid substitutions** of the Cox-1 molecule. None of the mutations were located near the catalytic site as judged from a three-dimensional model of the human Cox-L Carriers and non-carriers of one of the mutations behaved similarly when **aggregation** and granule content release function were studied using collagen, ADP and arachidonic acid as agonists. Conclusion: The results do not support the hypothesis that common variants of the Cox-1 gene results in unblocked Cox-1 molecules in aspirin non-responders.

L32 ANSWER 4 OF 15 EMBASE on STN
2002402933 EMBASE New class of **inhibitors** of amyloid-β **fibril formation**: Implications for the mechanism of pathogenesis in Alzheimer's disease. Lashuel H.A.; Hartley D.M.; Balakhaneh D.; Aggarwal A.; Teichberg S.; Callaway D.J.E.. H.A. Lashuel, Brigham and Women's Hospital, Department of Neurology, Harvard Medical School, Cambridge, MA 02139, United States. hlashuel@hms.harvard.edu. Journal of Biological Chemistry 277/45 (42881-42890) 8 Nov 2002. Refs: 65.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB The amyloid hypothesis suggests that the process of amyloid-β protein (Aβ) fibrillogenesis is responsible for triggering a cascade of physiological events that contribute directly to the initiation and progression of Alzheimer's disease. Consequently, preventing this process might provide a viable therapeutic strategy for slowing and/or preventing the progression of this devastating disease. A promising strategy to achieve prevention of this disease is to discover compounds that inhibit Aβ polymerization and deposition. Herein, we describe a new class of small molecules that inhibit Aβ aggregation, which is based on the chemical structure of apomorphine. These molecules were found to interfere with Aβ1-40 fibrillization as determined by transmission electron microscopy, Thioflavin T fluorescence and velocity sedimentation analytical ultracentrifugation studies. Using electron microscopy, time-dependent studies demonstrate that apomorphine and its derivatives promote the oligomerization of Aβ but inhibit its fibrillization.

Preliminary structural activity studies demonstrate that the 10,11-dihydroxy substitutions of the D-ring of apomorphine are required for the inhibitory effectiveness of these aporphines, and methylation of these hydroxyl groups reduces their inhibitory potency. The ability of these small molecules to inhibit Aβ amyloid **fibril formation** appears to be linked to their tendency to undergo rapid autoxidation, suggesting that autoxidation product(s) acts directly or indirectly on Aβ and inhibits its fibrillization. The inhibitory properties of the compounds presented suggest a new class of small molecules that could serve as a scaffold for the design of more efficient **inhibitors** of Aβ amyloidogenesis in vivo.

L32 ANSWER 5 OF 15 MEDLINE on STN
2002662550. PubMed ID: 12403615. Disulfide-bond formation in the transthyretin mutant Y114C prevents amyloid **fibril formation** in vivo and in vitro. Eneqvist Therese; Olofsson Anders; Ando Yukio; Miyakawa Taihei; Katsuragi Shoichi; Jass Jana; Lundgren Erik; Sauer-Eriksson A Elisabeth. (Umea Centre for Molecular Pathogenesis and Department of Molecular Biology, Umea University, SE-901 87 Umea, Sweden.) Biochemistry, (2002 Nov 5) 41 (44) 13143-51. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The Y114C mutation in human transthyretin (TTR) is associated with a particular form of familial amyloidotic polyneuropathy. We show that vitreous aggregates ex vivo consist of either regular amyloid fibrils or disordered disulfide-linked precipitates that maintain the ability to bind Congo red. Furthermore, we demonstrate in vitro that the ATTR Y114C mutant exists in three forms: one unstable but nativelike tetrameric form, one highly aggregated form in which a network of disulfide bonds is formed, and one fibrillar form. The disulfide-linked aggregates and the fibrillar form of the mutant can be induced by heat induction under nonreduced and reduced conditions, respectively. Both forms are recognized by the amyloid specific antibody MAB(39-44). In a previous study, we have linked exposure of this epitope in TTR to a three-residue shift in beta-strand D. The X-ray crystallographic structure of reduced tetrameric ATTR Y114C shows a structure similar to that of the wild type but with a more buried position of Cys10 and with beta-mercaptoethanol associated with Cys114, verifying the strong tendency for this residue to form disulfide bonds. Combined with the ex vivo data, our in vitro findings suggest that ATTR Y114C can lead to disease either by forming regular unbranched amyloid fibrils or by forming disulfide-linked aggregates that maintain amyloid-like properties but are unable to form regular amyloid fibrils.

L32 ANSWER 6 OF 15 MEDLINE on STN
2000169140. PubMed ID: 10704169. Platelet GP IIIa Pl(A) polymorphisms display different sensitivities to agonists. Michelson A D; Furman M I; Goldschmidt-Clermont P; Mascelli M A; Hendrix C; Coleman L; Hamlington J; Barnard M R; Kickler T; Christie D J; Kundu S; Bray P F. (Departments of Medicine and Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.) Circulation, (2000 Mar 7) 101 (9) 1013-8. Journal code: 0147763. ISSN: 1524-4539. Pub. country: United States. Language: English.

AB BACKGROUND: Both inherited predisposition and platelet hyperreactivity have been associated with ischemic coronary events, but mechanisms that support genetic differences among platelets from different subjects are generally lacking. Associations between the platelet Pl(A2) polymorphism of GP IIIa and coronary syndromes raise the question as to whether this inherited variation may contribute to platelet hyperreactivity. **METHODS** AND RESULTS: In this study, we characterized functional parameters in platelets from healthy donors with the Pl(A) (HPA-1) polymorphism, a Leu (Pl(A1)) to Pro (Pl(A2)) substitution at position 33 of the GP IIIa subunit of the platelet GP IIb/IIIa receptor (integrin alpha(IIb)beta(3)). We studied 56 normal donors (20 Pl(A1,A1), 20 Pl(A1,A2), and 16 Pl(A2,A2)). Compared with Pl(A1,A1) platelets, Pl(A2)-positive platelets showed a gene dosage effect for significantly

greater surface-expressed P-selectin, GP IIb/IIIa-bound fibrinogen, and activated GP IIb/IIIa in response to low-dose ADP. Surface expression of GP IIb/IIIa was similar in resting platelets of all 3 genotypes but was significantly greater on Pl(A2,A2) platelets after ADP stimulation (P=0.003 versus Pl(A1,A1); P=0.03 versus Pl(A1,A2)). Pl(A1,A2) platelets were more sensitive to **inhibition** of **aggregation** by pharmacologically relevant concentrations of aspirin and abciximab. CONCLUSIONS: Pl(A2)-positive platelets displayed a lower threshold for activation, and platelets heterozygous for Pl(A) alleles showed increased sensitivity to 2 antiplatelet drugs. These in vitro platelet studies may have relevance for in vivo thrombotic conditions.

L32 ANSWER 7 OF 15 MEDLINE on STN

2001040337. PubMed ID: 11070162. Inhibition of amyloid fiber assembly by both BiP and its target peptide. Davis P D; Raffen R; Dul L J; Vogen M S; Williamson K E; Stevens J F; Argon Y. (Department of Pathology and Committee on Immunology, The University of Chicago, Illinois 60637, USA.) Immunity, (2000 Oct) 13 (4) 433-42. Journal code: 9432918. ISSN: 1074-7613. Pub. country: United States. Language: English.

AB Immunoglobulin light chain (LC) normally is a soluble, secreted protein, but some LC assemble into ordered fibrils whose deposition in tissues results in amyloidosis and organ failure. Here we reconstitute **fibril formation** in vitro and show that preformed fibrils can nucleate polymerization of soluble LC. This prion-like behavior has important physiological implications, since somatic mutations generate multiple related LC sequences. Furthermore, we demonstrate that **fibril formation** in vitro and aggregation of whole LC within cells are inhibited by BiP and by a synthetic peptide that is identical to a major LC binding site for BiP. We propose that LC form fibrils via an interprotein loop swap and that the underlying conformational change should be amenable to drug therapy.

L32 ANSWER 8 OF 15 EMBASE on STN

2000003859 EMBASE **Inhibition** of calpain blocks platelet secretion, **aggregation**, and spreading. Croce K.; Flaumenhaft R.; Rivers M.; Furie B.; Furie B.C.; Herman I.M.; Potter D.A.. D.A. Potter, Dept. of Medicine, New England Medical Center 245, 750 Washington St., Boston, MA 02111, United States. Journal of Biological Chemistry 274/51 (36321-36327) 17 Dec 1999.
Refs: 67.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Previous studies have indicated that the Ca2+-dependent protease, calpain, is activated in platelets within 30-60 s of thrombin stimulation, but specific roles of calpain in platelets remain to be identified. To directly test the functions of calpain during platelet activation, a novel strategy was developed for introducing calpain's specific biological **inhibitor**, calpastatin, into platelets prior to activation. This **method** involves treatment of platelets with a fusion peptide, calpastat, consisting of the cell-penetrating signal sequence from Kaposi's fibroblast growth factor connected to a calpain-**inhibiting** consensus sequence derived from calpastatin. Calpastat specifically **inhibits** thrombin peptide (SFLLR)-induced α-granule secretion (IC50 = 20 μM) during the first 30 s of activation, thrombin-induced platelet **aggregation** (IC50 = 50 μM), and platelet spreading on glass surfaces (IC50 = 34 μM). Calpastat-Ala, a mutant peptide in which alanine is substituted at conserved calpastatin residues, lacks calpain **inhibitory** activity and fails to **inhibit** secretion, **aggregation**, or spreading. The peptidyl calpain **inhibitors** calpeptin, MDL 28,170 (MDL) and E64d also **inhibit** secretion, **aggregation** and spreading, but require 3-10-fold higher concentrations than calpastat for biological activity. Together, these findings demonstrate that calpain regulates platelet secretion,

aggregation, and spreading and indicate that calpain plays an earlier role in platelet activation following thrombin receptor stimulation than had been previously detected.

L32 ANSWER 9 OF 15 MEDLINE on STN
1999007248. PubMed ID: 9789022. Inhibiting transthyretin conformational changes that lead to amyloid **fibril formation.**
Peterson S A; Klabunde T; Lashuel H A; Purkey H; Sacchettini J C; Kelly J W. (Department of Chemistry and Skaggs Institute of Chemical Biology, Scripps Research Institute, 10550 North Torrey Pines Road MB 12, La Jolla, CA 92037, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1998 Oct 27) 95 (22) 12956-60. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Insoluble protein fibrils resulting from the self-assembly of a conformational intermediate are implicated as the causative agent in several severe human amyloid diseases, including Alzheimer's disease, familial amyloid polyneuropathy, and senile systemic amyloidosis. The latter two diseases are associated with transthyretin (TTR) amyloid fibrils, which appear to form in the acidic partial denaturing environment of the lysosome. Here we demonstrate that flufenamic acid (Flu) inhibits the conformational changes of TTR associated with amyloid **fibril formation.** The crystal structure of TTR complexed with Flu demonstrates that Flu mediates intersubunit hydrophobic interactions and intersubunit hydrogen bonds that stabilize the normal tetrameric fold of TTR. A small-molecule **inhibitor** that stabilizes the normal conformation of a protein is desirable as a possible approach to treat amyloid diseases. Molecules such as Flu also provide the means to rigorously test the amyloid hypothesis, i.e., the apparent causative role of amyloid fibrils in amyloid disease.

L32 ANSWER 10 OF 15 MEDLINE on STN
1998234371. PubMed ID: 9565605. Instability of the amyloidogenic cystatin C variant of hereditary cerebral hemorrhage with amyloidosis, Icelandic type. Wei L; Berman Y; Castano E M; Cadene M; Beavis R C; Devi L; Levy E. (Departments of Pharmacology and Pathology, New York University Medical Center, New York, New York 10016, USA.) Journal of biological chemistry, (1998 May 8) 273 (19) 11806-14. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB A cystatin C variant with L68Q substitution and a truncation of 10 NH2-terminal residues is the major constituent of the amyloid deposited in the cerebral vasculature of patients with the Icelandic form of hereditary cerebral hemorrhage with amyloidosis (HCHWA-I). Variant and wild type cystatin C production, processing, secretion, and clearance were studied in human cell lines stably overexpressing the cystatin C genes. Immunoblot and mass spectrometry analyses demonstrated monomeric cystatin C in cell homogenates and culture media. While cystatin C formed concentration-dependent dimers, the HCHWA-I variant dimerized at lower concentrations than the wild type protein. Amino-terminal sequence analysis revealed that the variant and normal proteins produced and secreted are the full-length cystatin C. Pulse-chase experiments demonstrated similar levels of normal and variant cystatin C production and secretion. However, the secreted variant cystatin C exhibited an increased susceptibility to a serine protease in conditioned media and in human cerebrospinal fluid, explaining its depletion from the cerebrospinal fluid of HCHWA-I patients. Thus, the **amino acid substitution** may induce unstable cystatin C with intact inhibitory activity and predisposition to self-aggregation and amyloid **fibril formation.**

L32 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
1993:617409 Document No. 119:217409 Analogs of von Willebrand factor-binding peptides of glycoprotein GPIbα and their manufacture for use as antithrombotics. Ruggeri, Zaverio M.; Ware, Jerry L. (Scripps Research Institute, USA). PCT Int. Appl. WO 9316712 A1 19930902, 50 pp.
DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB,

HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1993-US1734 19930225. PRIORITY: US 1992-842077 19920226.

AB Peptides derived from glycoprotein GPIbα sequences that interact with von Willebrand factor are prepared for use as antithrombotics. These peptides have **amino acid substitutions** that increase their affinity for von Willebrand factor. The peptides are manufactured by expression of the coding sequence in an appropriate host. An analog of His1-Ala302 glycoprotein GP1α with Gly-233 was prepared by site-directed mutagenesis of the gene and the protein manufactured by expression in CHO-KI cells. Ristocetin-induced binding of 125I-von Willebrand factor to the novel peptide was measured by an enzyme-linked immunofiltration technique using conditioned medium as a source of the glycoprotein. The novel peptide showed binding to von Willebrand factor comparable to the wild type at high ristocetin concns. but the analog more efficient binding at low ristocetin concns. **Methods** for mutagenesis and screening for peptides with increased affinity are described.

L32 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1993:579192 Document No. 119:179192 **Inhibitors** of stem cell proliferation. Craig, Stewart; Hunter, Michael George; Edwards, Richard Mark; Czaplewski, Lloyd George; Gilbert, Richard James (British Bio-Technology Ltd., UK). PCT Int. Appl. WO 9313206 A1 19930708, 177 pp. DESIGNATED STATES: W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, UA, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1992-GB2390 19921223. PRIORITY: GB 1991-27319 19911223; GB 1992-21587 19921014.

AB Analogs of macrophage inflammatory protein MIP-1α and LD78 and ACT-2 are used as **inhibitors** of stem cell proliferation. These analogs show reduced self-**aggregation** and so have higher concns. of biol. active oligomers in solution in addition to improved solubility properties.

A synthetic gene for human LD78 with codon usage optimized for expression in Saccharomyces cerevisiae was prepared by standard **methods** and mutagenized by oligonucleotide-directed site-specific mutagenesis and the resulting genes expressed in yeast from the PGK promoter. The resulting analogs were screened for the extent of **aggregation** and their binding to the LD78 receptor studied. Receptor binding was less extensive for those analogs with lower **aggregation** states. The roles of individual amino acids in **aggregation** and in receptor binding were consistent with a tetrameric model for the protein with several of the amino acids involved in **aggregation** also involved in receptor binding. A mutant with near-normal receptor binding and low levels of **aggregation** was able to **inhibit** proliferation of hemopoietic stem cells (day 12 CFU-S cells) at 1.5 ng/mL. Manufacture of the proteins in S. cerevisiae and Pichia pastoris was demonstrated.

L32 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN

1993:225680 Document No. 118:225680 Therapeutic polypeptides based on von Willebrand factor. Chang, Michael N.; Mcgarry, Daniel G.; Regan, John R.; Ruggeri, Zaverio M.; Ware, Jerry L. (Rhone-Poulenc Rorer International (Holdings) Inc., USA; Scripps Research Institute). PCT Int. Appl. WO 9300357 A1 19930107, 174 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US5472 19920629. PRIORITY: US 1991-720588 19910628.

AB Polypeptide fragments of von Willebrand Factor (vWF) are disclosed which have affinity for the blood platelet glycoprotein GPIb receptor. The

polypeptides are produced by DNA mutagenesis and cloning methodol. The polypeptides are useful for the treatment of thrombosis. Also disclosed is an anionic oligomer with greater affinity for the polypeptides of the invention than for mature vWF. Thus a mutant vWH 441-773 subunit sequence with 7 Cys to Gly replacements (construction and expression described) was effective in a dose-dependent fashion in **inhibiting** vWF binding. Another mutant fragment, with 5 Cys to Gly replacements (leaving 2 Cys residues for intrachain disulfide bond formation) **inhibited** binding of an anti-GPIb monoclonal antibody. Other studies were consistent with the hypothesis that **amino acid substitutions** in the Cys 509-695 loop region of the mature vWF subunit are responsible for the mol. basis of Type IIb von Willebrand disease. It was further demonstrated that residue 550 in the mature vWF subunit has no direct effect on binding to GPIbα but is important in the context of modulating the structure of the vWF subunit and hence activity of the GPIbα binding region (residues 474-488 and 694-708). An improved **method** for solubilization of recombinant and disulfide-stabilized vWF fragments is also described.

L32 ANSWER 14 OF 15 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
91:546910 The Genuine Article (R) Number: GH605. A MUTATION IN THE AMYLOID PRECURSOR PROTEIN ASSOCIATED WITH HEREDITARY ALZHEIMERS-DISEASE. MURRELL J; FARLOW M; GHETTI B; BENSON M D (Reprint). INDIANA UNIV, SCH MED, DEPT MED, INDIANAPOLIS, IN, 46202; INDIANA UNIV, SCH MED, DEPT MED & MOLEC GENET, INDIANAPOLIS, IN, 46202; INDIANA UNIV, SCH MED, DEPT NEUROL, INDIANAPOLIS, IN, 46202; INDIANA UNIV, SCH MED, DEPT PATHOL, INDIANAPOLIS, IN, 46202; VET AFFAIRS RICHARD L ROUDEBUSH MED CTR, INDIANAPOLIS, IN, 46202. SCIENCE (1991) Vol. 254, No. 5028, pp. 97-99. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Alzheimer's disease is a form of localized amyloidosis characterized by cerebral cortical amyloid plaques, neurofibrillary tangles, and amyloid deposits within the walls of leptomeningeal vessels. Although most cases of Alzheimer's disease are sporadic, kindreds with autosomal-dominant inheritance of the syndrome suggest that a single mutation may be important in pathogenesis. Direct sequencing of DNA from a family with autopsy-proven Alzheimer's disease revealed a single **amino acid substitution** (Phe for Val) in the transmembrane domain of the amyloid precursor protein. This mutation correlates with the presence of Alzheimer's disease in all patients in this study, and may be the inherited factor causing both amyloid **fibril formation** and dementia.

L32 ANSWER 15 OF 15 MEDLINE on STN DUPLICATE 1
89235594. PubMed ID: 2541223. Stroke in Icelandic patients with hereditary amyloid angiopathy is related to a mutation in the cystatin C gene, an **inhibitor** of cysteine proteases. Levy E; Lopez-Otin C; Ghiso J; Geltner D; Frangione B. (Department of Pathology, New York University Medical Center, New York 10016.) Journal of experimental medicine, (1989 May 1) 169 (5) 1771-8. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.
AB Cystatin C is an **inhibitor** of lysosomal cysteine proteases and consists of 120 amino acids. A variant of cystatin C lacking the first NH2-terminal residues and having one **amino acid substitution** at position 68 forms amyloid deposits mainly in the walls of brain arteries, causing fatal strokes in Icelandic patients with familial cerebral hemorrhage secondary to a form of an autosomal dominant amyloidosis. To understand the molecular basis of the genetic defect, the gene encoding cystatin C was isolated from genomic DNA libraries made from normal tissue and the brain of an Icelandic patient with hereditary cerebral hemorrhage with amyloidosis (HCHWA-I). The data indicate that the cystatin C gene encodes a polypeptide of 146 amino acids, of which the first 26 correspond to a secretory peptide signal sequence. The gene contains two intervening sequences that interrupt the coding region at amino acids 55 and 93. Comparison with genes encoding salivary cystatins

and kininogen proteins show sequence homology and conservation of exon-intron structure. Except for a mutation in the second exon (CAG instead of CTG in the normal gene, resulting in the substitution of glutamine for a leucine residue), the gene cloned from the brain of the Icelandic patient is identical to the normal cystatin C gene. Thus, HCHWA-I is the first familial type of amyloidosis related to a point mutation in a gene encoding for an **inhibitor.** The mutation in the structural gene encoding cystatin C appears to be the primary defect in this inherited disorder causing amyloid **fibril formation** and accumulation followed by cerebral hemorrhage.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	290.84	291.05

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-23.56	-23.56

STN INTERNATIONAL LOGOFF AT 17:55:51 ON 02 JUN 2004